

AEOMICA-12

FOUR HUMAN ZINC-FINGER-CONTAINING PROTEINS:

MDZ3, MDZ4, MDZ7 AND MDZ12

REFERENCE TO SEQUENCE LISTING SUBMITTED ON COMPACT DISC

5 The present application includes a Sequence
Listing filed on one CD-R disc, provided in duplicate,
containing a single file named sequence.txt, having
1,048,233 bytes, last modified on August 2, 2001 and
recorded August 2, 2001. The Sequence Listing contained in
said file on said disc is incorporated herein by reference
10 in its entirety.

FIELD OF THE INVENTION

The present invention relates to four novel human
zinc-finger-containing proteins: MDZ3, MDZ4, MDZ7 and MDZ12,
and a splice variant of MDZ12, MDZ12b (the original MDZ12 is
15 here named MDZ12a for this reason). More specifically, the
invention provides isolated nucleic acid molecules encoding
MDZ3, MDZ4, MDZ7, MDZ12, fragments thereof, vectors and host
cells comprising isolated nucleic acid molecules encoding

MDZ3, MDZ4, MDZ7 and MDZ12; MDZ3, MDZ4, MDZ7 and MDZ12 polypeptides, antibodies, transgenic cells and non-human organisms, and diagnostic, therapeutic, and investigational methods of using the same.

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BACKGROUND OF THE INVENTION

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Zinc-finger (ZNF)-containing genes collectively represent the largest family of sequence-specific nucleic acid binding regulatory proteins in mammalian genomes, with 10 600-1000 family members estimated for the human genome (Hoovers et al., *Genomics* 12:254-263 (1992)). Most ZNF-containing proteins are thought to act as DNA-binding regulators of transcription, either activating or repressing 15 expression of specific genes (El-Baradi and Pieler, *Mech. Dev.* 35:155-169 (1991)). However, a few family members have been found to bind specifically to RNA (e.g. ZNF74; Grondin et al., *J. Biol. Chem.* 271:15458-15467 (1996)).

The most common form of the ZNF motif, termed 20 Kruppel or C2H2-type, was first described as a repeated domain in the *Xenopus* transcription factor TFIIIA (Miller et al., *EMBO J.* 4:1609-1614 (1985), but is named after a similar domain found in the *Drosophila* Kruppel protein, which also functions as a transcription factor (Rosenberg et 25 al., *Nature* 319:336-339 (1986)). Kruppel (C2H2)-type ZNF motifs share a common backbone polypeptide sequence of $Cx_2Cx_3Fx_5Lx_2Hx_3H$, where x can be any amino acid. ZNF motifs may be found dispersed throughout a polypeptide or may be clustered into one or more tandemly repeated blocks. In 30 cases where the repeats are arranged in tandem, adjacent motifs are typically joined by a highly conserved stretch of seven amino acids (TGEKPYX, with X being any amino acid) termed the H-C link (Schuh et al., *Cell* 47:1025-1032

(1986)). The conserved cysteine (C) and histidine (H) residues collectively coordinate a single zinc ion, with the intervening amino acids forming an alpha helical structure that is responsible for specific binding to a nucleotide triplet. Among proteins with multiple, clustered ZNF motifs, each repeated unit binds to a different, but consecutive, nucleotide triplet (Suzuki et al., *Nucl. Acids Res.* 22:3397-3405 (1994)). Thus, for proteins with multiple ZNF repeats, it is the concerted activity of most or all of the repeat units that determines the proteins' DNA binding properties and ultimate biological function.

Another hallmark of ZNF proteins is that they frequently contain additional functional domains that further define their biological activities. One motif that is commonly found in conjunction with ZNF motifs in mammalian proteins is the Kruppel-associated box (KRAB) motif, first described by Bellefroid et al. *Proc. Natl. Acad. Sci. USA* 88:3608-3612 (1991). This motif can be divided into two sub-elements referred to as KRAB A and B domains. However, not all proteins contain both A and B domains (Bellefroid et al., *EMBO J.* 12:1363-1374 (1993)). Several studies have demonstrated that the KRAB A domain acts as a repressor of transcription (Margolin et al., *Proc. Natl. Acad. Sci. USA* 91:4509-4513 (1994); Witzgall et al., *Proc. Natl. Acad. Sci. USA* 91:4515-4518 (1994); Pengue et al., *Nucl. Acids Res.* 22:2908-2914 (1994)). The KRAB B domain by itself does not convey strong transcriptional repressor function, but may enhance the activity of an associated KRAB A domain (Margolin et al., *Proc. Natl. Acad. Sci. USA* 91:4509-4513 (1994)). A general mechanism for transcriptional repression appears to be shared by all members of the KRAB subfamily of ZNF proteins, and involves the recruitment of the common co-repressor protein

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KAP1/KRIP-1/TIF-beta by KRAB-ZNF proteins to sites of gene repression (Friedman *et al.*, *Genes Devel.* 10:2067-2078 (1996); Kim *et al.*, *Proc. Natl. Acad. Sci. USA* 93:15299-15304 (1996); Moosmann *et al.*, *Nucl. Acids Res.* 24:4849-4867 (1996)).

A second conserved motif that is often found in Kruppel (C2H2)-type ZNF proteins is called the SCAN box or LeR (leucine-rich) domain (Yokoyama *et al.*, *Biochim. Biophys. Acta.* 1353:13-17 (1997); Williams *et al.*, *Mol. Cell Biol.* 19:8526-8535 (1999)). The SCAN box functions primarily as an oligomerization domain that allows self-association of proteins or association between different proteins containing compatible SCAN boxes (Williams *et al.*, *Mol. Cell Biol.* 19:8526-8535 (1999)). Typically, the SCAN box is located at the N-terminus of the protein while the ZNF motifs are clustered near its C-terminus. In contrast to the KRAB domain, the SCAN box does not have a direct activator or repressor role in specific gene transcription.

ZNF proteins have been implicated in a wide range of biological activities that includes regulation of cell proliferation and differentiation as well as controlling patterns of embryonic development. For example, mouse Krox20 is a ZNF protein that is expressed specifically in the hindbrain, and disruption of the gene by mutation results in abnormalities in brain development and death shortly after birth (Swiatek and Gridley, *Genes Devel.* 7:2071-2084 (1993)). Another ZNF protein, human SALL1 has been shown to be the gene responsible for Townes-Brocks syndrome, an autosomal-dominant disorder characterized by abnormalities in the development of ears, limbs and kidneys (Kohlhase *et al.*, *Nature Genet.* 18:81-83 (1998)).

ZNF proteins have also been implicated in tumorigenesis in humans. For example, the product of the

Wilms' Tumor gene (WT1), Kruppel-type ZNF protein, is normally expressed during kidney development. However, mutations that affect the DNA binding ZNF motifs can result in embryonal renal neoplasia (Call *et al.*, *Cell* 60:509-520 (1990)). Mutations in other ZNF genes have also been shown to be the causative agents in a number of other cancers, including acute promyelocytic leukemia (Chen *et al.*, *EMBO J.* 12:1161-1167 (1993); Shaknovich *et al.*, *Mol. Cell Biol.* 18:5533-5545 (1998)) and t(8;13) leukemia/lymphoma syndrome (Xiao *et al.*, *Nature Genet.* 18:84-87 (1998)).

Recent reports suggest that at least one-third, and likely a higher percentage, of human genes are alternatively spliced. Hanke *et al.*, *Trends Genet.* 15(1):389 - 390 (1999); Mironov *et al.*, *Genome Res.* 9:1288-93 (1999); Brett *et al.*, *FEBS Lett.* 474(1):83-6 (2000). Alternative splicing has been proposed to account for at least part of the difference between the number of genes recently called from the completed human genome draft sequence - 30,000 to 40,000 (Genome International Sequencing Consortium, *Nature* 409:860-921 (15 February 2001) - and earlier predictions of human gene number that routinely ranged as high as 120,000, Liang *et al.*, *Nature Genet.* 25(2):239-240 (2000). With the *Drosophila* homolog of one human gene reported to have 38,000 potential alternatively spliced variants, Schmucker *et al.*, *Cell* 101:671 (2000), it now appears that alternative splicing may permit the relatively small number of human coding regions to encode millions, perhaps tens of millions, of structurally distinct proteins and protein isoforms.

Given the demonstrated roles of ZNF proteins in cell proliferation, differentiation and development, in combination with tumorigenesis when expressed aberrantly, there is a need to identify and to characterize human genes

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that encode ZNF-containing proteins. Given the importance of alternative splicing in providing further tissue-specific and developmental regulation of human proteins, there is a need to identify and to characterize splice variants of ZNF-
5 containing proteins.

SUMMARY OF THE INVENTION

10 The present invention solves these and other needs in the art by providing isolated nucleic acids that encode four novel human zinc-finger-containing proteins -- MDZ3, MDZ4, MDZ7 and MDZ12a -- as well as a splice variant of MDZ12a, termed MDZ12b, and fragments thereof.

15 MDZ3 encodes a protein with a SCAN box and a more divergent KRAB domain, in addition to 7 ZNF motifs. MDZ4 encodes a protein with SCAN box domain in addition to 5 ZNF motifs. MDZ7 encodes a protein that contains 7 ZNF motifs and no additional known elements. MDZ12 encodes a protein
20 with a divergent and possibly partial form of the KRAB motif (containing only the B domain) along with 12 ZNF repeats.

We have also isolated a splice variant of MDZ12, designated MDZ12b (and have thus denominated the originally isolated transcript MDZ12a), which is expressed in some of
25 the tissues tested. MDZ12b may encode a protein with 12 ZNF repeats (termed MDZ12bL) if the internal initiation methionine is used for protein translation, otherwise it encodes a 44 amino acid peptide (termed MDZ12bS) due to the introduction of a stop codon in the inserted exon.

30 In other aspects, the invention provides vectors for propagating and expressing the nucleic acids of the present invention, host cells comprising the nucleic acids and vectors of the present invention, proteins, protein

fragments, and protein fusions of any of the four novel human zinc-Finger-containing proteins: MDZ3, MDZ4, MDZ7, MDZ12a and MDZ12b (S and L), and antibodies thereto.

The invention further provides pharmaceutical
5 formulations of the nucleic acids, proteins, and antibodies of the present invention.

In other aspects, the invention provides transgenic cells and non-human organisms comprising nucleic acids of any one or more of four novel human zinc-finger-
10 containing genes -- MDZ3, MDZ4, MDZ7, MDZ12a and MDZ12b -- and transgenic cells and non-human organisms with targeted disruption of the endogenous orthologue of any of the four novel human Zinc-Finger-containing genes, MDZ3, MDZ4, MDZ7, MDZ12a and MDZ12b.

15 The invention additionally provides diagnostic, investigational, and therapeutic methods based on the nucleic acids, proteins, antibodies, mimetics, agonists, and antagonists of the present invention.

20 BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects and advantages of the present invention will be apparent upon consideration of the following detailed description taken in conjunction with the
25 accompanying drawings, in which like characters refer to like parts throughout, and in which:

FIGS. 1A - 1C schematize the protein domain structure of MDZ3, including the overall structure of MDZ3 and the alignment of SCAN box and KRAB domain in MDZ3 with
30 similar motifs;

FIG. 2 is a map showing the genomic structure of MDZ3 encoded at chromosome 7q22.1;

FIG. 3 presents the nucleotide and predicted amino acid sequences of MDZ3;

FIGS. 4A and 4B schematize the protein domain structure of MDZ4, including the overall structure of MDZ4 and the alignment of the SCAN box in MDZ4 with similar motifs;

FIG. 5 is a map showing the genomic structure of MDZ4 encoded at chromosome 6p21.3-22.2;

FIG. 6 presents the nucleotide and predicted amino acid sequences of MDZ4;

FIG. 7 schematizes the protein domain structure of MDZ7;

FIG. 8 is a map showing the genomic structure of MDZ7 encoded at chromosome 16p11.2;

FIG. 9 presents the nucleotide and predicted amino acid sequences of MDZ7;

FIGS. 10A and 10B schematize the protein domain structure of MDZ12, including the overall structure of MDZ12a and MDZ12bL (the longer ORF generated using the internal translation initiation site) and the alignment of the KRAB domain in MDZ12a with similar motifs;

FIG. 11 is a map showing the genomic structure of MDZ12a and MDZ12b encoded at chromosome 15q26.1;

FIG. 12 presents the nucleotide and predicted amino acid sequences of MDZ12a;

FIG. 13 presents the nucleotide and predicted amino acid sequences of MDZ12b;

FIG. 14 presents the RT-PCR analysis of the MDZ3 gene expression;

FIG. 15 presents the RT-PCR analysis of the MDZ7 gene expression; and

FIG. 16 presents the RT-PCR analysis of the MDZ12 gene expression.

DETAILED DESCRIPTION OF THE INVENTION

5 Mining the sequence of the human genome for novel human genes, the present inventors have identified four novel human zinc-finger-containing genes: MDZ3, MDZ4, MDZ7 and MDZ12 (a and b). Each of the four genes acts as a sequence-specific nucleic acid binding regulatory protein, and plays a role in cell proliferation, differentiation and
10 development. When expressed aberrantly, the genes contribute to neoplasia.

Detailed Description Of The MDZ3 Gene

15 As schematized in FIG. 1, the protein product of the newly isolated MDZ3 gene shares certain protein domains and an overall structural organization with a family of other zinc-finger proteins. The shared structural features strongly imply that MDZ3 plays a role similar to that of the
20 SCAN box- and KRAB motif-containing Kruppel family zinc-finger proteins as a sequence-specific nucleic acid binding regulatory protein, and is likely to participate in protein-protein interactions with other transcription modulators. Thus, MDZ3 is a clinically useful diagnostic marker and
25 potential therapeutic agent for a variety of diseases, including developmental disorders and cancer.

In common with the other family members of the SCAN box containing Kruppel zinc-finger proteins, MDZ3 has a SCAN box near the N-terminus, which has been shown to
30 participate in protein-protein interactions. The C-terminal region of the MDZ3 protein contains seven copies of C2H2 zinc fingers. There is also weak homology to KRAB domain in the middle of MDZ3.

FIG. 2 shows the genomic organization of MDZ3.

At the top is shown approximately 14 kb of the 177 kb bacterial artificial chromosome (BAC), with GenBank accession number, that spans the MDZ3 locus.

5 As shown in FIG. 2, MDZ3, encoding a protein of 544 amino acids, comprises exons 1 - 8. Predicted molecular weight of the protein, prior to any post-translational modification, is 61.4 kD.

As further discussed in the examples herein,
10 expression of MDZ3 was assessed using RT-PCR analysis. RT-PCR product for MDZ3 was clearly produced from brain, testis, heart and bone marrow, but not from lung, liver, or skeletal muscle.

15 Detailed Description Of The MDZ4 Gene

As schematized in FIG. 4, the product of the newly isolated MDZ4 gene shares certain protein domains and an overall structural organization with a family of zinc finger
20 proteins. The shared structural features strongly imply that MDZ4 plays a role similar to those SCAN box-containing Kruppel family zinc-finger proteins as a potential transcription regulator, and is likely to participate in protein-protein interactions with other transcription
25 modulators. Thus, MDZ4 is a clinically useful diagnostic marker and potential therapeutic agent for a variety of diseases, including developmental disorders and cancer.

In common with the other family members of the SCAN box-containing Kruppel zinc-finger proteins, MDZ4 has a
30 SCAN box near the N-terminus, which has been shown to participate in protein-protein interactions. The C-terminal region of the MDZ4 protein contains five copies of C2H2 zinc fingers.

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FIG. 5 shows the genomic organization of MDZ4.

At the top is shown about 9 kb of the 128 kb P1 artificial chromosome (PAC) (with GenBank accession number), that spans the MDZ4 locus. The genome-derived single-exon probe first used to demonstrate expression from this locus, is shown below the PAC and is labeled "500"; the 500 bp probe includes sequence drawn from exon two as well as flanking intron two.

As shown in FIG. 5, MDZ4, encoding a protein of 389 amino acids, is comprised of exons 1 - 4. Predicted molecular weight, prior to any post-translational modification, is 44.9 kD.

As further discussed in the examples herein, expression of MDZ4 was assessed using hybridization to genome-derived single exon microarrays. Microarray analysis of exons 2 and 3 showed expression in all tissues tested, including bone marrow, brain, heart, hela, adult liver, fetal liver, lung, placenta and prostate.

Detailed Description Of The MDZ7 Gene

As schematized in FIG. 7, the newly isolated MDZ7 gene product is mainly composed of seven tandemly arrayed Kruppel-type (C2H2) zinc finger repeats. Such a structure implies that MDZ7 is likely to function in sequence-specific DNA binding and impart a regulatory effect on specific gene expression. Thus, MDZ7 is a clinically useful diagnostic marker and potential therapeutic agent for a variety of diseases, including developmental disorders and cancer.

FIG. 8 shows the genomic organization of MDZ7.

At the top is shown about 5.5 kb of the 121 kb bacterial artificial chromosome (BAC), with GenBank accession number, that spans the MDZ7 locus.

As shown in FIG. 8, MDZ7 is comprised of four exons and encodes a protein of 248 amino acids. Predicted molecular weight of the MDZ7 protein, prior to any post-translational modification, is 72.0 kD.

As further discussed in the examples herein, expression of MDZ7 was assessed using RT-PCR. RT-PCR analysis of MDZ7 showed expression only in testes, but not in brain, lung, liver, kidney, skeletal muscle, heart, whole fetus, or Hela cells.

Detailed Description Of The MDZ12 Gene

As schematized in FIG. 10A, and further shown in FIG. 11, the newly isolated MDZ12 encodes a MDZ12a isoform and potentially two other isoforms, which we designate 12bL (for "long"), and 12bS (for "short"); FIG. 10A shows the 12bL isoform. MDZ12a contains a partial KRAB motif as well as twelve C2H2 zinc fingers. MDZ12bL encodes a protein with 12 C2H2 zinc fingers. Such features strongly imply that MDZ12a (and MDZ12bL, if translated) plays a role as a potential transcription regulator, and is likely to participate in protein-protein interactions with other transcription modulators. Thus MDZ12 is a clinically useful diagnostic marker and potential therapeutic agent for a variety of diseases, including developmental disorders and cancer.

FIG. 11 shows the genomic organization of MDZ12.

At the top is shown a portion of the 173 kb bacterial artificial chromosome (BAC), with GenBank accession number, that spans the MDZ12 locus. The genome-derived single-exon probe first used to demonstrate expression from this locus includes sequence drawn solely from exon 4 of MDZ12a.

As shown in FIG. 11, MDZ12a encodes a protein of 483 amino acids, comprising exons 1 - 4. Predicted molecular weight of the protein, prior to any post-translational modification, is 55.1 kD. The inclusion of a novel exon between exons 2 and 3 introduces an inframe stop codon in MDZ12b, and thus MDZ12b encodes a short polypeptide of 44 amino acids (MDZ12bS). The use of an internal methionine as initiation methionine in MDZ12b could potentially further encode a 332 amino acid protein (MDZ12bL). The predicted molecular weight of the MDZ12bL protein, prior to any post-translational modification, is 38.2 kD.

As further discussed in the examples herein, expression of MDZ12 was assessed using RT-PCR. The abundance of PCR product indicates that MDZ12a is expressed in all tissues examined, with highest expression in brain, heart, skeletal muscle, testis and Hela cells. MDZ12b, however, is expressed with lower to much lower abundance compared with MDZ12a in bone marrow, brain, heart, kidney, placenta, skeletal muscle, testis and Hela cells with almost no expression in liver.

As more fully described below, the present invention provides isolated nucleic acids that encode MDZ3, MDZ4, MDZ7, MDZ12a and MDZ12b and fragments thereof. The invention further provides vectors for propagation and expression of the nucleic acids of the present invention, host cells comprising the nucleic acids and vectors of the present invention, proteins, protein fragments, and protein fusions of the present invention, and antibodies specific for all or any one of the isoforms. The invention provides pharmaceutical formulations of the nucleic acids, proteins, and antibodies of the present invention. The invention further provides transgenic cells and non-human organisms

comprising human MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids,
and transgenic cells and non-human organisms with targeted
disruption of the endogenous orthologue of the human MDZ3,
MDZ4, MDZ7 or MDZ12. The invention additionally provides
5 diagnostic, investigational, and therapeutic methods based
on the MDZ3, MDZ4, MDZ7, MDZ12 nucleic acids, proteins, and
antibodies of the present invention.

DEFINITIONS

10

Unless defined otherwise, all technical and
scientific terms used herein have the meaning commonly
understood by one of ordinary skill in the art to which this
invention belongs.

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As used herein, **"nucleic acid"** (synonymously,
"polynucleotide") includes polynucleotides having natural
nucleotides in native 5'-3' phosphodiester linkage - e.g.,
DNA or RNA - as well as polynucleotides that have nonnatural
nucleotide analogues, nonnative internucleoside bonds, or
20 both, so long as the nonnatural polynucleotide is capable of
sequence-discriminating basepairing under experimentally
desired conditions. Unless otherwise specified, the term
"nucleic acid" includes any topological conformation; the
term thus explicitly comprehends single-stranded, double-
25 stranded, partially duplexed, triplexed, hairpinned,
circular, and padlocked conformations.

As used herein, an **"isolated nucleic acid"** is a
nucleic acid molecule that exists in a physical form that is
nonidentical to any nucleic acid molecule of identical
30 sequence as found in nature; "isolated" does not require,
although it does not prohibit, that the nucleic acid so
described has itself been physically removed from its native
environment.

For example, a nucleic acid can be said to be "isolated" when it includes nucleotides and/or internucleoside bonds not found in nature. When instead composed of natural nucleosides in phosphodiester linkage, a nucleic acid can be said to be "isolated" when it exists at a purity not found in nature, where purity can be adjudged with respect to the presence of nucleic acids of other sequence, with respect to the presence of proteins, with respect to the presence of lipids, or with respect the presence of any other component of a biological cell, or when the nucleic acid lacks sequence that flanks an otherwise identical sequence in an organism's genome, or when the nucleic acid possesses sequence not identically present in nature.

As so defined, "isolated nucleic acid" includes nucleic acids integrated into a host cell chromosome at a heterologous site, recombinant fusions of a native fragment to a heterologous sequence, recombinant vectors present as episomes or as integrated into a host cell chromosome.

As used herein, an isolated nucleic acid "encodes" a reference polypeptide when at least a portion of the nucleic acid, or its complement, can be directly translated to provide the amino acid sequence of the reference polypeptide, or when the isolated nucleic acid can be used, alone or as part of an expression vector, to express the reference polypeptide *in vitro*, in a prokaryotic host cell, or in a eukaryotic host cell.

As used herein, the term "**exon**" refers to a nucleic acid sequence found in genomic DNA that is bioinformatically predicted and/or experimentally confirmed to contribute contiguous sequence to a mature mRNA transcript.

As used herein, the phrase **"open reading frame"** and the equivalent acronym **"ORF"** refer to that portion of a transcript-derived nucleic acid that can be translated in its entirety into a sequence of contiguous amino acids. As so defined, an ORF has length, measured in nucleotides, exactly divisible by 3. As so defined, an ORF need not encode the entirety of a natural protein.

As used herein, the phrase **"ORF-encoded peptide"** refers to the predicted or actual translation of an ORF.

As used herein, the phrase **"degenerate variant"** of a reference nucleic acid sequence intends all nucleic acid sequences that can be directly translated, using the standard genetic code, to provide an amino acid sequence identical to that translated from the reference nucleic acid sequence.

As used herein, the term **"microarray"** and the equivalent phrase **"nucleic acid microarray"** refer to a substrate-bound collection of plural nucleic acids, hybridization to each of the plurality of bound nucleic acids being separately detectable. The substrate can be solid or porous, planar or non-planar, unitary or distributed.

As so defined, the term **"microarray"** and phrase **"nucleic acid microarray"** include all the devices so called in Schena (ed.), DNA Microarrays: A Practical Approach (Practical Approach Series), Oxford University Press (1999) (ISBN: 0199637768); *Nature Genet.* 21(1)(suppl):1 - 60 (1999); and Schena (ed.), Microarray Biochip: Tools and Technology, Eaton Publishing Company/BioTechniques Books Division (2000) (ISBN: 1881299376), the disclosures of which are incorporated herein by reference in their entireties.

As so defined, the term **"microarray"** and phrase **"nucleic acid microarray"** also include substrate-bound

collections of plural nucleic acids in which the plurality of nucleic acids are distributably disposed on a plurality of beads, rather than on a unitary planar substrate, as is described, *inter alia*, in Brenner *et al.*, *Proc. Natl. Acad. Sci. USA* 97(4):166501670 (2000), the disclosure of which is incorporated herein by reference in its entirety; in such case, the term "microarray" and phrase "nucleic acid microarray" refer to the plurality of beads in aggregate.

As used herein with respect to solution phase hybridization, the term "**probe**", or equivalently, "**nucleic acid probe**" or "**hybridization probe**", refers to an isolated nucleic acid of known sequence that is, or is intended to be, detectably labeled. As used herein with respect to a nucleic acid microarray, the term "probe" (or equivalently "**nucleic acid probe**" or "**hybridization probe**") refers to the isolated nucleic acid that is, or is intended to be, bound to the substrate. In either such context, the term "target" refers to nucleic acid intended to be bound to probe by sequence complementarity.

As used herein, the expression "**probe comprising SEQ ID NO:X**", and variants thereof, intends a nucleic acid probe, at least a portion of which probe has either (i) the sequence directly as given in the referenced SEQ ID NO:X, or (ii) a sequence complementary to the sequence as given in the referenced SEQ ID NO:X, the choice as between sequence directly as given and complement thereof dictated by the requirement that the probe be complementary to the desired target.

As used herein, the phrases "**expression of a probe**" and "**expression of an isolated nucleic acid**" and their linguistic equivalents intend that the probe or, (respectively, the isolated nucleic acid), or a probe (or, respectively, isolated nucleic acid) complementary in

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sequence thereto, can hybridize detectably under high stringency conditions to a sample of nucleic acids that derive from mRNA transcripts from a given source. For example, and by way of illustration only, expression of a probe in "liver" means that the probe can hybridize detectably under high stringency conditions to a sample of nucleic acids that derive from mRNA obtained from liver.

As used herein, **"a single exon probe"** comprises at least part of an exon ("reference exon") and can hybridize detectably under high stringency conditions to transcript-derived nucleic acids that include the reference exon. The single exon probe will not, however, hybridize detectably under high stringency conditions to nucleic acids that lack the reference exon and that instead consist of one or more exons that are found adjacent to the reference exon in the genome.

For purposes herein, **"high stringency conditions"** are defined for solution phase hybridization as aqueous hybridization (i.e., free of formamide) in 6X SSC (where 20X SSC contains 3.0 M NaCl and 0.3 M sodium citrate), 1% SDS at 65°C for at least 8 hours, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. **"Moderate stringency conditions"** are defined for solution phase hybridization as aqueous hybridization (i.e., free of formamide) in 6X SSC, 1% SDS at 65°C for at least 8 hours, followed by one or more washes in 2x SSC, 0.1% SDS at room temperature.

For microarray-based hybridization, standard "high stringency conditions" are defined as hybridization in 50% formamide, 5X SSC, 0.2 µg/µl poly(dA), 0.2 µg/µl human cot1 DNA, and 0.5% SDS, in a humid oven at 42°C overnight, followed by successive washes of the microarray in 1X SSC, 0.2% SDS at 55°C for 5 minutes, and then 0.1X SSC, 0.2% SDS,

at 55°C for 20 minutes. For microarray-based hybridization, "moderate stringency conditions", suitable for cross-hybridization to mRNA encoding structurally- and functionally-related proteins, are defined to be the same as those for high stringency conditions but with reduction in temperature for hybridization and washing to room temperature (approximately 25°C).

As used herein, the terms **"protein"**, **"polypeptide"**, and **"peptide"** are used interchangeably to refer to a naturally-occurring or synthetic polymer of amino acid monomers (residues), irrespective of length, where amino acid monomer here includes naturally-occurring amino acids, naturally-occurring amino acid structural variants, and synthetic non-naturally occurring analogs that are capable of participating in peptide bonds. The terms **"protein"**, **"polypeptide"**, and **"peptide"** explicitly permits of post-translational and post-synthetic modifications, such as glycosylation.

The term **"oligopeptide"** herein denotes a protein, polypeptide, or peptide having 25 or fewer monomeric subunits.

The phrases **"isolated protein"**, **"isolated polypeptide"**, **"isolated peptide"** and **"isolated oligopeptide"** refer to a protein (or respectively to a polypeptide, peptide, or oligopeptide) that is nonidentical to any protein molecule of identical amino acid sequence as found in nature; "isolated" does not require, although it does not prohibit, that the protein so described has itself been physically removed from its native environment.

For example, a protein can be said to be "isolated" when it includes amino acid analogues or derivatives not found in nature, or includes linkages other than standard peptide bonds.

When instead composed entirely of natural amino acids linked by peptide bonds, a protein can be said to be "isolated" when it exists at a purity not found in nature — where purity can be adjudged with respect to the presence of proteins of other sequence, with respect to the presence of non-protein compounds, such as nucleic acids, lipids, or other components of a biological cell, or when it exists in a composition not found in nature, such as in a host cell that does not naturally express that protein.

A **"purified protein"** (equally, a purified polypeptide, peptide, or oligopeptide) is an isolated protein, as above described, present at a concentration of at least 95%, as measured on a weight basis with respect to total protein in a composition. A **"substantially purified protein"** (equally, a substantially purified polypeptide, peptide, or oligopeptide) is an isolated protein, as above described, present at a concentration of at least 70%, as measured on a weight basis with respect to total protein in a composition.

As used herein, the phrase **"protein isoforms"** refers to a plurality of proteins having nonidentical primary amino acid sequence but that share amino acid sequence encoded by at least one common exon.

As used herein, the phrase **"alternative splicing"** and its linguistic equivalents includes all types of RNA processing that lead to expression of plural protein isoforms from a single gene; accordingly, the phrase **"splice variant(s)"** and its linguistic equivalents embraces mRNAs transcribed from a given gene that, however processed, collectively encode plural protein isoforms. For example, and by way of illustration only, splice variants can include exon insertions, exon extensions, exon truncations, exon deletions, alternatives in the 5' untranslated region ("5'

UT") and alternatives in the 3' untranslated region ("3' UT"). Such 3' alternatives include, for example, differences in the site of RNA transcript cleavage and site of poly(A) addition. See, e.g., Gautheret et al., *Genome Res.* 8:524-530 (1998).

As used herein, "**orthologues**" are separate occurrences of the same gene in multiple species. The separate occurrences have similar, albeit nonidentical, amino acid sequences, the degree of sequence similarity depending, in part, upon the evolutionary distance of the species from a common ancestor having the same gene.

As used herein, the term "**paralogues**" indicates separate occurrences of a gene in one species. The separate occurrences have similar, albeit nonidentical, amino acid sequences, the degree of sequence similarity depending, in part, upon the evolutionary distance from the gene duplication event giving rise to the separate occurrences.

As used herein, the term "**homologues**" is generic to "orthologues" and "paralogues".

As used herein, the term "**antibody**" refers to a polypeptide, at least a portion of which is encoded by at least one immunoglobulin gene, or fragment thereof, and that can bind specifically to a desired target molecule. The term includes naturally-occurring forms, as well as fragments and derivatives.

Fragments within the scope of the term "antibody" include those produced by digestion with various proteases, those produced by chemical cleavage and/or chemical dissociation, and those produced recombinantly, so long as the fragment remains capable of specific binding to a target molecule. Among such fragments are Fab, Fab', Fv, F(ab)'₂, and single chain Fv (scFv) fragments.

Derivatives within the scope of the term include antibodies (or fragments thereof) that have been modified in sequence, but remain capable of specific binding to a target molecule, including: interspecies chimeric and humanized
5 antibodies; antibody fusions; heteromeric antibody complexes and antibody fusions, such as diabodies (bispecific antibodies), single-chain diabodies, and intrabodies (see, e.g., Marasco (ed.), Intracellular Antibodies: Research and Disease Applications, Springer-Verlag New York, Inc. (1998)
10 (ISBN: 3540641513), the disclosure of which is incorporated herein by reference in its entirety).

As used herein, antibodies can be produced by any known technique, including harvest from cell culture of native B lymphocytes, harvest from culture of hybridomas,
15 recombinant expression systems, and phage display.

As used herein, "antigen" refers to a ligand that can be bound by an antibody; an antigen need not itself be immunogenic. The portions of the antigen that make contact with the antibody are denominated "epitopes".

20 "Specific binding" refers to the ability of two molecular species concurrently present in a heterogeneous (inhomogeneous) sample to bind to one another in preference to binding to other molecular species in the sample. Typically, a specific binding interaction will discriminate
25 over adventitious binding interactions in the reaction by at least two-fold, more typically by at least 10-fold, often at least 100-fold; when used to detect analyte, specific binding is sufficiently discriminatory when determinative of the presence of the analyte in a heterogeneous
30 (inhomogeneous) sample. Typically, the affinity or avidity of a specific binding reaction is least about 10^{-7} M, with specific binding reactions of greater specificity typically

having affinity or avidity of at least 10^{-8} M to at least about 10^{-9} M.

As used herein, "**molecular binding partners**" – and equivalently, "**specific binding partners**" – refer to pairs
5 of molecules, typically pairs of biomolecules, that exhibit specific binding. Nonlimiting examples are receptor and ligand, antibody and antigen, and biotin to any of avidin, streptavidin, neutrAvidin and captAvidin.

The term "**antisense**", as used herein, refers to a
10 nucleic acid molecule sufficiently complementary in sequence, and sufficiently long in that complementary sequence, as to hybridize under intracellular conditions to (i) a target mRNA transcript or (ii) the genomic DNA strand complementary to that transcribed to produce the target mRNA
15 transcript.

The term "**portion**", as used with respect to nucleic acids, proteins, and antibodies, is synonymous with "fragment".

20 NUCLEIC ACID MOLECULES

In a first aspect, the invention provides isolated nucleic acids that encode MDZ3, MDZ4, MDZ7 or MDZ12, variants having at least 65% sequence identity thereto,
25 degenerate variants thereof, variants that encode MDZ3, MDZ4, MDZ7 or MDZ12 proteins having conservative or moderately conservative substitutions, cross-hybridizing nucleic acids, and fragments thereof.

FIGS. 3, 6, 9, 12 and 13 present the nucleotide
30 sequences of the MDZ3, MDZ4, MDZ7, MDZ12a and MDZ12b cDNA clones, respectively, with predicted amino acid translations. The sequences are further presented in the Sequence Listing, incorporated herein by reference in its

entirety, in SEQ ID NOs: 1 (full length nucleotide sequence of human MDZ3 cDNA), 3 (full length amino acid sequence of MDZ3), 3027 (full length nucleotide sequence of human MDZ4 cDNA), 3029 (full length amino acid sequence of MDZ4), 4407 (full length nucleotide sequence of human MDZ7 cDNA), 4409 (full length amino acid sequence of MDZ7), 5770 (full length nucleotide sequence of human MDZ12a cDNA), 5772 (full length amino acid sequence of MDZ12a), 6938 (full length nucleotide sequence of human MDZ12b cDNA), 6939 (full length amino acid sequence of MDZ12bS) and 6940 (full length amino acid sequence of MDZ12bL).

Unless otherwise indicated, each nucleotide sequence is set forth herein as a sequence of deoxyribonucleotides. It is intended, however, that the given sequence be interpreted as would be appropriate to the polynucleotide composition: for example, if the isolated nucleic acid is composed of RNA, the given sequence intends ribonucleotides, with uridine substituted for thymidine.

Unless otherwise indicated, nucleotide sequences of the isolated nucleic acids of the present invention were determined by sequencing a DNA molecule that had resulted, directly or indirectly, from at least one enzymatic polymerization reaction (e.g., reverse transcription and/or polymerase chain reaction) using an automated sequencer (such as the MegaBACE™ 1000, Molecular Dynamics, Sunnyvale, CA, USA), or by reliance upon such sequence or upon genomic sequence prior-accessioned into a public database. Unless otherwise indicated, all amino acid sequences of the polypeptides of the present invention were predicted by translation from the nucleic acid sequences so determined.

As a consequence, any nucleic acid sequence presented herein may contain errors introduced by erroneous incorporation of nucleotides during polymerization, by

erroneous base calling by the automated sequencer (although such sequencing errors have been minimized for the nucleic acids directly determined herein, unless otherwise indicated, by the sequencing of each of the complementary strands of a duplex DNA), or by similar errors accessioned into the public database.

Accordingly, the MDZ3, MDZ4, MDZ7, MDZ12a and MDZ12b cDNA clones described herein have been separately deposited in a public repository (American Type Culture Collection, Manassas, Virginia, USA, "ATCC"). Each of the MDZ3, MDZ4, MDZ7 cDNA clones was sent for deposit to ATCC in a discrete tube on August 1, 2001 and received by ATCC August 2, 2001, and respectively accorded accession numbers of _____, _____, _____. The two splice variants of the MDZ12 gene - MDZ12a and MDZ12b - were sent for deposit in admixture in a single tube to ATCC on August 1, 2001, received at ATCC on August 2, 2001, and jointly accorded the single accession number _____. Any errors in sequence reported herein can be determined and corrected by sequencing nucleic acids propagated from the deposited clones using standard techniques.

Single nucleotide polymorphisms (SNPs) occur frequently in eukaryotic genomes - more than 1.4 million SNPs have already identified in the human genome, International Human Genome Sequencing Consortium, *Nature* 409:860 - 921 (2001) - and the sequence determined from one individual of a species may differ from other allelic forms present within the population. Additionally, small deletions and insertions, rather than single nucleotide polymorphisms, are not uncommon in the general population, and often do not alter the function of the protein.

Accordingly, it is an aspect of the present invention to provide nucleic acids not only identical in

sequence to those described with particularity herein, but also to provide isolated nucleic acids at least about 65% identical in sequence to those described with particularity herein, typically at least about 70%, 75%, 80%, 85%, or 90% identical in sequence to those described with particularity herein, usefully at least about 91%, 92%, 93%, 94%, or 95% identical in sequence to those described with particularity herein, usefully at least about 96%, 97%, 98%, or 99% identical in sequence to those described with particularity herein, and, most conservatively, at least about 99.5%, 99.6%, 99.7%, 99.8% and 99.9% identical in sequence to those described with particularity herein. These sequence variants can be naturally occurring or can result from human intervention, as by random or directed mutagenesis.

For purposes herein, percent identity of two nucleic acid sequences is determined using the procedure of Tatiana et al., "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", *FEMS Microbiol Lett.* 174:247-250 (1999), which procedure is effectuated by the computer program BLAST 2 SEQUENCES, available online at

<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>.

To assess percent identity of nucleic acids, the BLASTN module of BLAST 2 SEQUENCES is used with default values of (i) reward for a match: 1; (ii) penalty for a mismatch: -2; (iii) open gap 5 and extension gap 2 penalties; (iv) gap X_dropoff 50 expect 10 word size 11 filter, and both sequences are entered in their entireties.

As is well known, the genetic code is degenerate, with each amino acid except methionine translated from a plurality of codons, thus permitting a plurality of nucleic acids of disparate sequence to encode the identical protein.

As is also well known, codon choice for optimal expression varies from species to species. The isolated nucleic acids of the present invention being useful for expression of MDZ3, MDZ4, MDZ7 or MDZ12 proteins and protein fragments, it is, therefore, another aspect of the present invention to provide isolated nucleic acids that encode MDZ3, MDZ4, MDZ7 or MDZ12 proteins and portions thereof not only identical in sequence to those described with particularity herein, but degenerate variants thereof as well.

As is also well known, amino acid substitutions occur frequently among natural allelic variants, with conservative substitutions often occasioning only *de minimis* change in protein function.

Accordingly, it is an aspect of the present invention to provide nucleic acids not only identical in sequence to those described with particularity herein, but also to provide isolated nucleic acids that encode MDZ3, MDZ4, MDZ7 or MDZ12, and portions thereof, having conservative amino acid substitutions, and also to provide isolated nucleic acids that encode MDZ3, MDZ4, MDZ7 or MDZ12, and portions thereof, having moderately conservative amino acid substitutions.

Although there are a variety of metrics for calling conservative amino acid substitutions, based primarily on either observed changes among evolutionarily related proteins or on predicted chemical similarity, for purposes herein a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix reproduced herein below (see Gonnet et al., *Science* 256(5062):1443-5 (1992)):

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
	A	2	-1	0	0	0	0	0	-1	-1	-1	0	-1	-2	0	1	1	-4	-2	0
	R	-1	5	0	0	-2	2	0	-1	1	-2	-2	3	-2	-3	-1	0	0	-2	-2
	N	0	0	4	2	-2	1	1	0	1	-3	-3	1	-2	-3	-1	1	0	-4	-1
5	D	0	0	2	5	-3	1	3	0	0	-4	-4	0	-3	-4	-1	0	0	-5	-3
	C	0	-2	-2	-3	12	-2	-3	-2	-1	-1	-2	-3	-1	-1	-3	0	0	-1	0
	Q	0	2	1	1	-2	3	2	-1	1	-2	-2	2	-1	-3	0	0	0	-3	-2
	E	0	0	1	3	-3	2	4	-1	0	-3	-3	1	-2	-4	0	0	0	-4	-3
	G	0	-1	0	0	-2	-1	-1	7	-1	-4	-4	-1	-4	-5	-2	0	-1	-4	-4
10	H	-1	1	1	0	-1	1	0	-1	6	-2	-2	1	-1	0	-1	0	0	-1	2
	I	-1	-2	-3	-4	-1	-2	-3	-4	-2	4	3	-2	2	1	-3	-2	-1	-2	-1
	L	-1	-2	-3	-4	-2	-2	-3	-4	-2	3	4	-2	3	2	-2	-2	-1	-1	0
	K	0	3	1	0	-3	2	1	-1	1	-2	-2	3	-1	-3	-1	0	0	-4	-2
	M	-1	-2	-2	-3	-1	-1	-2	-4	-1	2	3	-1	4	2	-2	-1	-1	-1	0
15	F	-2	-3	-3	-4	-1	-3	-4	-5	0	1	2	-3	2	7	-4	-3	-2	4	5
	P	0	-1	-1	-1	-3	0	0	-2	-1	-3	-2	-1	-2	-4	8	0	0	-5	-3
	S	1	0	1	0	0	0	0	0	-2	-2	0	-1	-3	0	2	2	-3	-2	-1
	T	1	0	0	0	0	0	0	-1	0	-1	-1	0	-1	-2	0	2	2	-4	-2
	W	-4	-2	-4	-5	-1	-3	-4	-4	-1	-2	-1	-4	-1	4	-5	-3	-4	14	4
20	Y	-2	-2	-1	-3	0	-2	-3	-4	2	-1	0	-2	0	5	-3	-2	-2	4	8
	V	0	-2	-2	-3	0	-2	-2	-3	-2	3	2	-2	2	0	-2	-1	0	-3	-1

For purposes herein, a "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix reproduced herein above.

As is also well known in the art, relatedness of nucleic acids can also be characterized using a functional test, the ability of the two nucleic acids to base-pair to one another at defined hybridization stringencies.

It is, therefore, another aspect of the invention to provide isolated nucleic acids not only identical in sequence to those described with particularity herein, but also to provide isolated nucleic acids ("cross-hybridizing nucleic acids") that hybridize under high stringency conditions (as defined herein below) to all or to a portion of various of the isolated MDZ3, MDZ4, MDZ7 or MDZ12 nucleic

acids of the present invention ("reference nucleic acids"),
as well as cross-hybridizing nucleic acids that hybridize
under moderate stringency conditions to all or to a portion
of various of the isolated MDZ3, MDZ4, MDZ7 or MDZ12 nucleic
5 acids of the present invention.

Such cross-hybridizing nucleic acids are useful,
inter alia, as probes for, and to drive expression of,
proteins related to the proteins of the present invention as
alternative isoforms, homologues, paralogues, and
10 orthologues. Particularly useful orthologues are those from
other primate species, such as chimpanzee, rhesus macaque,
monkey, baboon, orangutan, and gorilla; from rodents, such
as rats, mice, guinea pigs; from lagomorphs, such as
rabbits; and from domestic livestock, such as cow, pig,
15 sheep, horse, goat and chickens.

For purposes herein, high stringency conditions
are defined as aqueous hybridization (*i.e.*, free of
formamide) in 6X SSC (where 20X SSC contains 3.0 M NaCl and
0.3 M sodium citrate), 1% SDS at 65°C for at least 8 hours,
20 followed by one or more washes in 0.2X SSC, 0.1% SDS at
65°C. For purposes herein, moderate stringency conditions
are defined as aqueous hybridization (*i.e.*, free of
formamide) in 6X SSC, 1% SDS at 65°C for at least 8 hours,
followed by one or more washes in 2x SSC, 0.1% SDS at room
25 temperature.

The hybridizing portion of the reference nucleic
acid is typically at least 15 nucleotides in length, often
at least 17 nucleotides in length. Often, however, the
hybridizing portion of the reference nucleic acid is at
30 least 20 nucleotides in length, 25 nucleotides in length,
and even 30 nucleotides, 35 nucleotides, 40 nucleotides, and
50 nucleotides in length. Of course, cross-hybridizing
nucleic acids that hybridize to a larger portion of the

reference nucleic acid - for example, to a portion of at least 50 nt, at least 100 nt, at least 150 nt, 200 nt, 250 nt, 300 nt, 350 nt, 400 nt, 450 nt, or 500 nt or more - or even to the entire length of the reference nucleic acid, are also useful.

The hybridizing portion of the cross-hybridizing nucleic acid is at least 75% identical in sequence to at least a portion of the reference nucleic acid. Typically, the hybridizing portion of the cross-hybridizing nucleic acid is at least 80%, often at least 85%, 86%, 87%, 88%, 89% or even at least 90% identical in sequence to at least a portion of the reference nucleic acid. Often, the hybridizing portion of the cross-hybridizing nucleic acid will be at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical in sequence to at least a portion of the reference nucleic acid sequence. At times, the hybridizing portion of the cross-hybridizing nucleic acid will be at least 99.5% identical in sequence to at least a portion of the reference nucleic acid.

The invention also provides fragments of various of the isolated nucleic acids of the present invention.

By "fragments" of a reference nucleic acid is here intended isolated nucleic acids, however obtained, that have a nucleotide sequence identical to a portion of the reference nucleic acid sequence, which portion is at least 17 nucleotides and less than the entirety of the reference nucleic acid. As so defined, "fragments" need not be obtained by physical fragmentation of the reference nucleic acid, although such provenance is not thereby precluded.

In theory, an oligonucleotide of 17 nucleotides is of sufficient length as to occur at random less frequently than once in the three gigabase human genome, and thus to provide a nucleic acid probe that can uniquely identify the

reference sequence in a nucleic acid mixture of genomic complexity. As is well known, further specificity can be obtained by probing nucleic acid samples of subgenomic complexity, and/or by using plural fragments as short as 17 nucleotides in length collectively to prime amplification of nucleic acids, as, e.g., by polymerase chain reaction (PCR).

As further described herein below, nucleic acid fragments that encode at least 6 contiguous amino acids (i.e., fragments of 18 nucleotides or more) are useful in directing the expression or the synthesis of peptides that have utility in mapping the epitopes of the protein encoded by the reference nucleic acid. See, e.g., Geysen et al., "Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid," *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1984); and U.S. Pat. Nos. 4,708,871 and 5,595,915, the disclosures of which are incorporated herein by reference in their entireties.

As further described herein below, fragments that encode at least 8 contiguous amino acids (*i.e.*, fragments of 24 nucleotides or more) are useful in directing the expression or the synthesis of peptides that have utility as immunogens. See, *e.g.*, Lerner, "Tapping the immunological repertoire to produce antibodies of predetermined specificity," *Nature* 299:592-596 (1982); Shinnick *et al.*, "Synthetic peptide immunogens as vaccines," *Annu. Rev. Microbiol.* 37:425-46 (1983); Sutcliffe *et al.*, "Antibodies that react with predetermined sites on proteins," *Science* 219:660-6 (1983), the disclosures of which are incorporated herein by reference in their entireties.

30 The nucleic acid fragment of the present invention is thus at least 17 nucleotides in length, typically at least 18 nucleotides in length, and often at least 24 nucleotides in length. Often, the nucleic acid of the

intronic and/or intergenic sequence that is identically contiguous to the exon in the genome.

The minimum length of genome-derived single exon probes is defined by the requirement that the exonic portion
5 be of sufficient length to hybridize under high stringency conditions to transcript-derived nucleic acids.

Accordingly, the exon portion is at least 17 nucleotides, typically at least 18 nucleotides, 20 nucleotides, 24
10 nucleotides, 25 nucleotides or even 30, 35, 40, 45, or 50 nucleotides in length, and can usefully include the entirety of the exon, up to 100 nt, 150 nt, 200 nt, 250 nt, 300 nt, 350 nt, 400 nt or even 500 nt or more in length.

The maximum length of genome-derived single exon probes is defined by the requirement that the probes contain
15 portions of no more than one exon, that is, be unable to hybridize detectably under high stringency conditions to nucleic acids that lack the reference exon but include one or more exons that are found adjacent to the reference exon the genome.

Given variable spacing of exons through eukaryotic
20 genomes, the maximum length of single exon probes of the present invention is typically no more than 25 kb, often no more than 20 kb, 15 kb, 10 kb or 7.5 kb, or even no more than 5 kb, 4 kb, 3 kb, or even no more than about 2.5 kb in
25 length.

The genome-derived single exon probes of the present invention can usefully include at least a first terminal priming sequence not found in contiguity with the rest of the probe sequence in the genome, and often will
30 contain a second terminal priming sequence not found in contiguity with the rest of the probe sequence in the genome.

The present invention also provides isolated genome-derived nucleic acids that include nucleic acid sequence elements that control transcription of the MDZ3, MDZ4, MDZ7 or MDZ12 gene.

5 With a complete draft of the human genome now available, genomic sequences that are within the vicinity of the MDZ3, MDZ4, MDZ7 or MDZ12 coding region (and that are additional to those described with particularity herein) can readily be obtained by PCR amplification.

10 The isolated nucleic acids of the present invention can be composed of natural nucleotides in native 5'-3' phosphodiester internucleoside linkage - e.g., DNA or RNA - or can contain any or all of nonnatural nucleotide analogues, nonnative internucleoside bonds, or post-
15 synthesis modifications, either throughout the length of the nucleic acid or localized to one or more portions thereof.

 As is well known in the art, when the isolated nucleic acid is used as a hybridization probe, the range of such nonnatural analogues, nonnative internucleoside bonds,
20 or post-synthesis modifications will be limited to those that permit sequence-discriminating basepairing of the resulting nucleic acid. When used to direct expression or RNA or protein *in vitro* or *in vivo*, the range of such nonnatural analogues, nonnative internucleoside bonds, or
25 post-synthesis modifications will be limited to those that permit the nucleic acid to function properly as a polymerization substrate. When the isolated nucleic acid is used as a therapeutic agent, the range of such changes will be limited to those that do not confer toxicity upon the
30 isolated nucleic acid.

 For example, when desired to be used as probes, the isolated nucleic acids of the present invention can usefully include nucleotide analogues that incorporate

Haptens that are commonly conjugated to nucleotides for subsequent labeling include biotin (biotin-11-dUTP, Molecular Probes, Inc., Eugene, OR, USA; biotin-21-UTP, biotin-21-dUTP, Clontech Laboratories, Inc., Palo Alto, CA, USA), digoxigenin (DIG-11-dUTP, alkali labile, DIG-11-UTP, Roche Diagnostics Corp., Indianapolis, IN, USA), and dinitrophenyl (dinitrophenyl-11-dUTP, Molecular Probes, Inc., Eugene, OR, USA).

As another example, when desired to be used for antisense inhibition of transcription or translation, the isolated nucleic acids of the present invention can usefully include altered, often nuclease-resistant, internucleoside bonds. See Hartmann *et al.* (eds.), Manual of Antisense Methodology (Perspectives in Antisense Science), Kluwer Law International (1999) (ISBN:079238539X); Stein *et al.* (eds.), Applied Antisense Oligonucleotide Technology, Wiley-Liss (cover (1998) (ISBN: 0471172790); Chadwick *et al.* (eds.), Oligonucleotides as Therapeutic Agents - Symposium No. 209, John Wiley & Son Ltd (1997) (ISBN: 0471972797), the disclosures of which are incorporated herein by reference in their entirety. Such altered internucleoside bonds are often desired also when the isolated nucleic acid of the present invention is to be used for or for targeted gene correction, Gamper *et al.*, *Nucl. Acids Res.* 28(21):4332-4339 (2000), the disclosures of which are incorporated herein by reference in its entirety.

Modified oligonucleotide backbones often preferred when the nucleic acid is to be used for antisense purposes are, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including

3'-amino phosphoramidate and aminoalkylphosphoramidates,
thionophosphoramidates, thionoalkylphosphonates,
thionoalkylphosphotriesters, and boranophosphates having
normal 3'-5' linkages, 2'-5' linked analogs of these, and
5 those having inverted polarity wherein the adjacent pairs of
nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to
5'-2'. Representative U.S. patents that teach the
preparation of the above phosphorus-containing linkages
include, but are not limited to, U.S. Pat. Nos. 3,687,808;
10 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897;
5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131;
5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677;
5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111;
5,563,253; 5,571,799; 5,587,361; and 5,625,050, the
15 disclosures of which are incorporated herein by reference in
their entireties.

Preferred modified oligonucleotide backbones for
antisense use that do not include a phosphorus atom have
backbones that are formed by short chain alkyl or cycloalkyl
20 internucleoside linkages, mixed heteroatom and alkyl or
cycloalkyl internucleoside linkages, or one or more short
chain heteroatomic or heterocyclic internucleoside linkages.

These include those having morpholino linkages (formed in
part from the sugar portion of a nucleoside); siloxane
25 backbones; sulfide, sulfoxide and sulfone backbones;
formacetyl and thioformacetyl backbones; methylene
formacetyl and thioformacetyl backbones; alkene containing
backbones; sulfamate backbones; methyleneimino and
methylenehydrazino backbones; sulfonate and sulfonamide
30 backbones; amide backbones; and others having mixed N, O, S
and CH₂ component parts. Representative U.S. patents that
teach the preparation of the above backbones include, but
are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315;

5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562;
5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967;
5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240;
5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704;
5 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439,
the disclosures of which are incorporated herein by
reference in their entireties.

In other preferred oligonucleotide mimetics, both
the sugar and the internucleoside linkage are replaced with
10 novel groups, such as peptide nucleic acids (PNA).

In PNA compounds, the phosphodiester backbone of
the nucleic acid is replaced with an amide-containing
backbone, in particular by repeating N-(2-aminoethyl)
glycine units linked by amide bonds. Nucleobases are bound
15 directly or indirectly to aza nitrogen atoms of the amide
portion of the backbone, typically by methylene carbonyl
linkages.

The uncharged nature of the PNA backbone provides
PNA/DNA and PNA/RNA duplexes with a higher thermal stability
20 than is found in DNA/DNA and DNA/RNA duplexes, resulting
from the lack of charge repulsion between the PNA and DNA or
RNA strand. In general, the T_m of a PNA/DNA or PNA/RNA
duplex is 1°C higher per base pair than the T_m of the
corresponding DNA/DNA or DNA/RNA duplex (in 100 mM NaCl).

25 The neutral backbone also allows PNA to form
stable DNA duplexes largely independent of salt
concentration. At low ionic strength, PNA can be hybridized
to a target sequence at temperatures that make DNA
hybridization problematic or impossible. And unlike DNA/DNA
30 duplex formation, PNA hybridization is possible in the
absence of magnesium. Adjusting the ionic strength,
therefore, is useful if competing DNA or RNA is present in

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the sample, or if the nucleic acid being probed contains a high level of secondary structure.

PNA also demonstrates greater specificity in binding to complementary DNA. A PNA/DNA mismatch is more destabilizing than DNA/DNA mismatch. A single mismatch in mixed a PNA/DNA 15-mer lowers the T_m by 8-20°C (15°C on average). In the corresponding DNA/DNA duplexes, a single mismatch lowers the T_m by 4-16°C (11°C on average). Because PNA probes can be significantly shorter than DNA probes, their specificity is greater.

Additionally, nucleases and proteases do not recognize the PNA polyamide backbone with nucleobase sidechains. As a result, PNA oligomers are resistant to degradation by enzymes, and the lifetime of these compounds is extended both *in vivo* and *in vitro*. In addition, PNA is stable over a wide pH range.

Because its backbone is formed from amide bonds, PNA can be synthesized using a modified peptide synthesis protocol. PNA oligomers can be synthesized by both Fmoc and tBoc methods. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is hereby incorporated herein by reference; automated PNA synthesis is readily achievable on commercial synthesizers (see, e.g., "PNA User's Guide," Rev. 2, February 1998, Perseptive Biosystems Part No. 60138, Applied Biosystems, Inc., Foster City, CA).

PNA chemistry and applications are reviewed, *inter alia*, in Ray et al., *FASEB J.* 14(9):1041-60 (2000); Nielsen et al., *Pharmacol Toxicol.* 86(1):3-7 (2000); Larsen et al., *Biochim Biophys Acta.* 1489(1):159-66 (1999); Nielsen, *Curr. Opin. Struct. Biol.* 9(3):353-7 (1999), and Nielsen, *Curr.*

Opin. Biotechnol. 10(1):71-5 (1999), the disclosures of which are incorporated herein by reference in their entireties.

Differences from nucleic acid compositions found in nature - e.g., nonnative bases, altered internucleoside linkages, post-synthesis modification - can be present throughout the length of the nucleic acid or can, instead, usefully be localized to discrete portions thereof. As an example of the latter, chimeric nucleic acids can be synthesized that have discrete DNA and RNA domains and demonstrated utility for targeted gene repair, as further described in U.S. Pat. Nos. 5,760,012 and 5,731,181, the disclosures of which are incorporated herein by reference in their entireties. As another example, chimeric nucleic acids comprising both DNA and PNA have been demonstrated to have utility in modified PCR reactions. See Misra *et al.*, *Biochem.* 37: 1917-1925 (1998); see also Finn *et al.*, *Nucl. Acids Res.* 24: 3357-3363 (1996), incorporated herein by reference.

Unless otherwise specified, nucleic acids of the present invention can include any topological conformation appropriate to the desired use; the term thus explicitly comprehends, among others, single-stranded, double-stranded, triplexed, quadruplexed, partially double-stranded, partially-triplexed, partially-quadruplexed, branched, hairpinned, circular, and padlocked conformations. Padlock conformations and their utilities are further described in Banér *et al.*, *Curr. Opin. Biotechnol.* 12:11-15 (2001); Escude *et al.*, *Proc. Natl. Acad. Sci. USA* 14;96(19):10603-7 (1999); Nilsson *et al.*, *Science* 265(5181):2085-8 (1994), the disclosures of which are incorporated herein by reference in their entireties. Triplex and quadruplex conformations, and their utilities, are reviewed in Praseuth *et al.*, *Biochim.*

Biophys. Acta. 1489(1):181-206 (1999); Fox, *Curr. Med. Chem.* 7(1):17-37 (2000); Kochetkova et al., *Methods Mol. Biol.* 130:189-201 (2000); Chan et al., *J. Mol. Med.* 75(4):267-82 (1997), the disclosures of which are incorporated herein by
5 reference in their entireties.

The nucleic acids of the present invention can be detectably labeled.

Commonly-used labels include radionuclides, such as ^{32}P , ^{33}P , ^{35}S , ^3H (and for NMR detection, ^{13}C and ^{15}N),
10 haptens that can be detected by specific antibody or high affinity binding partner (such as avidin), and fluorophores.

As noted above, detectable labels can be incorporated by inclusion of labeled nucleotide analogues in the nucleic acid. Such analogues can be incorporated by
15 enzymatic polymerization, such as by nick translation, random priming, polymerase chain reaction (PCR), terminal transferase tailing, and end-filling of overhangs, for DNA molecules, and *in vitro* transcription driven, e.g., from phage promoters, such as T7, T3, and SP6, for RNA molecules.

20 Commercial kits are readily available for each such labeling approach.

Analogues can also be incorporated during automated solid phase chemical synthesis.

As is well known, labels can also be incorporated
25 after nucleic acid synthesis, with the 5' phosphate and 3' hydroxyl providing convenient sites for post-synthetic covalent attachment of detectable labels.

Various other post-synthetic approaches permit internal labeling of nucleic acids.

30 For example, fluorophores can be attached using a cisplatin reagent that reacts with the N7 of guanine residues (and, to a lesser extent, adenine bases) in DNA, RNA, and PNA to provide a stable coordination complex

between the nucleic acid and fluorophore label (Universal Linkage System) (available from Molecular Probes, Inc., Eugene, OR, USA and Amersham Pharmacia Biotech, Piscataway, NJ, USA); see Alers et al., *Genes, Chromosomes & Cancer*, Vol. 25, pp. 301 - 305 (1999); Jelsma et al., *J. NIH Res.* 5:82 (1994); Van Belkum et al., *BioTechniques* 16:148-153 (1994), incorporated herein by reference. As another example, nucleic acids can be labeled using a disulfide-containing linker (FastTag™ Reagent, Vector Laboratories, Inc., Burlingame, CA, USA) that is photo- or thermally coupled to the target nucleic acid using aryl azide chemistry; after reduction, a free thiol is available for coupling to a hapten, fluorophore, sugar, affinity ligand, or other marker.

Multiple independent or interacting labels can be incorporated into the nucleic acids of the present invention.

For example, both a fluorophore and a moiety that in proximity thereto acts to quench fluorescence can be included to report specific hybridization through release of fluorescence quenching, Tyagi et al., *Nature Biotechnol.* 14: 303-308 (1996); Tyagi et al., *Nature Biotechnol.* 16, 49-53 (1998); Sokol et al., *Proc. Natl. Acad. Sci. USA* 95: 11538-11543 (1998); Kostrikis et al., *Science* 279:1228-1229 (1998); Marras et al., *Genet. Anal.* 14: 151-156 (1999); U.S. Pat. Nos. 5,846,726, 5,925,517, 5,925,517, or to report exonucleotidic excision, U.S. Pat. No. 5,538,848; Holland et al., *Proc. Natl. Acad. Sci. USA* 88:7276-7280 (1991); Heid et al., *Genome Res.* 6(10):986-94 (1996); Kuimelis et al., *Nucleic Acids Symp Ser.* (37):255-6 (1997); U.S. Patent No. 5,723,591, the disclosures of which are incorporated herein by reference in their entireties.

So labeled, the isolated nucleic acids of the present invention can be used as probes, as further described below.

Nucleic acids of the present invention can also usefully be bound to a substrate. The substrate can porous or solid, planar or non-planar, unitary or distributed; the bond can be covalent or noncovalent. Bound to a substrate, nucleic acids of the present invention can be used as probes in their unlabeled state.

10 For example, the nucleic acids of the present invention can usefully be bound to a porous substrate, commonly a membrane, typically comprising nitrocellulose, nylon, or positively-charged derivatized nylon; so attached, the nucleic acids of the present invention can be used to
15 detect MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids present within a labeled nucleic acid sample, either a sample of genomic nucleic acids or a sample of transcript-derived nucleic acids, *e.g.* by reverse dot blot.

The nucleic acids of the present invention can also usefully be bound to a solid substrate, such as glass, although other solid materials, such as amorphous silicon, crystalline silicon, or plastics, can also be used. Such plastics include polymethylacrylic, polyethylene, polypropylene, polyacrylate, polymethylmethacrylate, polyvinylchloride, polytetrafluoroethylene, polystyrene, polycarbonate, polyacetal, polysulfone, celluloseacetate, cellulosenitrate, nitrocellulose, or mixtures thereof.

Typically, the solid substrate will be rectangular, although other shapes, particularly disks and even spheres, present certain advantages. Particularly advantageous alternatives to glass slides as support substrates for array of nucleic acids are optical discs, as described in Demers, "Spatially Addressable Combinatorial

Chemical Arrays in CD-ROM Format," international patent publication WO 98/12559, incorporated herein by reference in its entirety.

5 The nucleic acids of the present invention can be attached covalently to a surface of the support substrate or applied to a derivatized surface in a chaotropic agent that facilitates denaturation and adherence by presumed noncovalent interactions, or some combination thereof.

10 The nucleic acids of the present invention can be bound to a substrate to which a plurality of other nucleic acids are concurrently bound, hybridization to each of the plurality of bound nucleic acids being separately detectable. At low density, e.g. on a porous membrane, these substrate-bound collections are typically denominated
15 macroarrays; at higher density, typically on a solid support, such as glass, these substrate bound collections of plural nucleic acids are colloquially termed microarrays. As used herein, the term microarray includes arrays of all densities. It is, therefore, another aspect of the
20 invention to provide microarrays that include the nucleic acids of the present invention.

The isolated nucleic acids of the present invention can be used as hybridization probes to detect, characterize, and quantify MDZ3, MDZ4, MDZ7 or MDZ12 nucleic
25 acids in, and isolate MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids from, both genomic and transcript-derived nucleic acid samples. When free in solution, such probes are typically, but not invariably, detectably labeled; bound to a substrate, as in a microarray, such probes are typically,
30 but not invariably unlabeled.

For example, the isolated nucleic acids of the present invention can be used as probes to detect and characterize gross alterations in the MDZ3, MDZ4, MDZ7 or

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MDZ12 genomic locus, such as deletions, insertions,
translocations, and duplications of the MDZ3, MDZ4, MDZ7 or
MDZ12 genomic locus through fluorescence *in situ*
hybridization (FISH) to chromosome spreads. See, e.g.,
5 Andreeff et al. (eds.), Introduction to Fluorescence In Situ
Hybridization: Principles and Clinical Applications, John
Wiley & Sons (1999) (ISBN: 0471013455), the disclosure of
which is incorporated herein by reference in its entirety.
The isolated nucleic acids of the present invention can be
10 used as probes to assess smaller genomic alterations using,
e.g., Southern blot detection of restriction fragment length
polymorphisms. The isolated nucleic acids of the present
invention can be used as probes to isolate genomic clones
that include the nucleic acids of the present invention,
15 which thereafter can be restriction mapped and sequenced to
identify deletions, insertions, translocations, and
substitutions (single nucleotide polymorphisms, SNPs) at the
sequence level.

The isolated nucleic acids of the present
20 invention can also be used as probes to detect,
characterize, and quantify MDZ3, MDZ4, MDZ7 or MDZ12 nucleic
acids in, and isolate MDZ3, MDZ4, MDZ7 or MDZ12 nucleic
acids from, transcript-derived nucleic acid samples.

For example, the isolated nucleic acids of the
25 present invention can be used as hybridization probes to
detect, characterize by length, and quantify MDZ3, MDZ4,
MDZ7 or MDZ12 mRNA by northern blot of total or poly-A⁺-
selected RNA samples. For example, the isolated nucleic
acids of the present invention can be used as hybridization
30 probes to detect, characterize by location, and quantify
MDZ3, MDZ4, MDZ7 or MDZ12 message by *in situ* hybridization
to tissue sections (see, e.g., Schwarchzacher et al., In
Situ Hybridization, Springer-Verlag New York (2000) (ISBN:

0387915966), the disclosure of which is incorporated herein by reference in its entirety). For example, the isolated nucleic acids of the present invention can be used as hybridization probes to measure the representation of MDZ3, MDZ4, MDZ7 or MDZ12 clones in a cDNA library. For example, the isolated nucleic acids of the present invention can be used as hybridization probes to isolate MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids from cDNA libraries, permitting sequence level characterization of MDZ3, MDZ4, MDZ7 or MDZ12 messages, including identification of deletions, insertions, truncations - including deletions, insertions, and truncations of exons in alternatively spliced forms - and single nucleotide polymorphisms.

All of the aforementioned probe techniques are well within the skill in the art, and are described at greater length in standard texts such as Sambrook et al., Molecular Cloning: A Laboratory Manual (3rd ed.), Cold Spring Harbor Laboratory Press (2001) (ISBN: 0879695773); Ausubel et al. (eds.), Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology (4th ed.), John Wiley & Sons, 1999 (ISBN: 047132938X); and Walker et al. (eds.), The Nucleic Acids Protocols Handbook, Humana Press (2000) (ISBN: 0896034593), the disclosures of which are incorporated herein by reference in their entirety.

As described in the Examples herein below, the nucleic acids of the present invention can also be used to detect and quantify MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids in transcript-derived samples - that is, to measure expression of the MDZ3, MDZ4, MDZ7 or MDZ12 gene - when included in a microarray. Measurement of MDZ3, MDZ4, MDZ7 or MDZ12 expression has particular utility in diagnosis and treatment of a variety of diseases, including developmental

As would be readily apparent to one of skill in the art, each MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acid probe -- whether labeled, substrate-bound, or both -- is thus currently available for use as a tool for measuring the level of MDZ3, MDZ4, MDZ7 or MDZ12 expression in each of the tissues in which expression has already been confirmed, notably testes for MDZ7, brain, testis, heart and bone marrow for MDZ3, bone marrow, brain, heart, hela, adult liver, fetal liver, lung, placenta and prostate for MDZ4, brain, heart, kidney, placenta, skeletal muscle, testis, Hela cells, bone marrow and liver for MDZ12. The utility is specific to the probe: under high stringency conditions, the probe reports the level of expression of message specifically containing that portion of the MDZ3, MDZ4, MDZ7 or MDZ12 gene included within the probe.

Measuring tools are well known in many arts, not just in molecular biology, and are known to possess credible, specific, and substantial utility. For example, U.S. Patent No. 6,016,191 describes and claims a tool for measuring characteristics of fluid flow in a hydrocarbon well; U.S. Patent No. 6,042,549 describes and claims a device for measuring exercise intensity; U.S. Patent No. 5,889,351 describes and claims a device for measuring viscosity and for measuring characteristics of a fluid; U.S. Patent No. 5,570,694 describes and claims a device for measuring blood pressure; U.S. Patent No. 5,930,143 describes and claims a device for measuring the dimensions of machine tools; U.S. Patent No. 5,279,044 describes and claims a measuring device for determining an absolute position of a movable element; U.S. Patent No. 5,186,042 describes and claims a device for measuring action force of

a wheel; and U.S. Patent No. 4,246,774 describes and claims a device for measuring the draft of smoking articles such as cigarettes.

As for tissues not yet demonstrated to express
5 MDZ3, MDZ4, MDZ7 or MDZ12, the MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acid probes of the present invention are currently available as tools for surveying such tissues to detect the presence of MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids.

Survey tools - i.e., tools for determining the
10 presence and/or location of a desired object by search of an area - are well known in many arts, not just in molecular biology, and are known to possess credible, specific, and substantial utility. For example, U.S. Patent No. 6,046,800 describes and claims a device for surveying an area for
15 objects that move; U.S. Patent No. 6,025,201 describes and claims an apparatus for locating and discriminating platelets from non-platelet particles or cells on a cell-by-cell basis in a whole blood sample; U.S. Patent No. 5,990,689 describes and claims a device for detecting and
20 locating anomalies in the electromagnetic protection of a system; U.S. Patent No. 5,984,175 describes and claims a device for detecting and identifying wearable user identification units; U.S. Patent No. 3,980,986 ("Oil well survey tool"), describes and claims a tool for finding the
25 position of a drill bit working at the bottom of a borehole.

As noted above, the nucleic acid probes of the present invention are useful in constructing microarrays; the microarrays, in turn, are products of manufacture that are useful for measuring and for surveying gene expression.

30 When included on a microarray, each MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acid probe makes the microarray specifically useful for detecting that portion of the MDZ3, MDZ4, MDZ7 or MDZ12 gene included within the probe, thus

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imparting upon the microarray device the ability to detect a signal where, absent such probe, it would have reported no signal. This utility makes each individual probe on such microarray akin to an antenna, circuit, firmware or software element included in an electronic apparatus, where the antenna, circuit, firmware or software element imparts upon the apparatus the ability newly and additionally to detect signal in a portion of the radio-frequency spectrum where previously it could not; such devices are known to have specific, substantial, and credible utility.

Changes in the level of expression need not be observed for the measurement of expression to have utility.

For example, where gene expression analysis is used to assess toxicity of chemical agents on cells, the failure of the agent to change a gene's expression level is evidence that the drug likely does not affect the pathway of which the gene's expressed protein is a part. Analogously, where gene expression analysis is used to assess side effects of pharmacologic agents - whether in lead compound discovery or in subsequent screening of lead compound derivatives - the inability of the agent to alter a gene's expression level is evidence that the drug does not affect the pathway of which the gene's expressed protein is a part.

WO 99/58720, incorporated herein by reference in its entirety, provides methods for quantifying the relatedness of a first and second gene expression profile and for ordering the relatedness of a plurality of gene expression profiles, without regard to the identity or function of the genes whose expression is used in the calculation.

Gene expression analysis, including gene expression analysis by microarray hybridization, is, of course, principally a laboratory-based art. Devices and

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apparatus used principally in laboratories to facilitate laboratory research are well-established to possess specific, substantial, and credible utility. For example, U.S. Patent No. 6,001,233 describes and claims a gel
5 electrophoresis apparatus having a cam-activated clamp; for example, U.S. Patent No. 6,051,831 describes and claims a high mass detector for use in time-of-flight mass spectrometers; for example, U.S. Patent NO. 5,824,269 describes and claims a flow cytometer—as is well known, few
10 gel electrophoresis apparatuses, TOF-MS devices, or flow cytometers are sold for consumer use.

Indeed, and in particular, nucleic acid microarrays, as devices intended for laboratory use in measuring gene expression, are well-established to have
15 specific, substantial and credible utility. Thus, the microarrays of the present invention have at least the specific, substantial and credible utilities of the microarrays claimed as devices and articles of manufacture in the following U.S. patents, the disclosures of each of
20 which is incorporated herein by reference: U.S. Patent Nos. 5,445,934 ("Array of oligonucleotides on a solid substrate"); 5,744,305 ("Arrays of materials attached to a substrate"); and 6,004,752 ("Solid support with attached molecules").

25 Genome-derived single exon probes and genome-derived single exon probe microarrays have the additional utility, *inter alia*, of permitting high-throughput detection of splice variants of the nucleic acids of the present invention, as further described in copending and commonly
30 owned U.S. Patent application no. 09/632,366, filed August 3, 2000, the disclosure of which is incorporated herein by reference in its entirety.

The isolated nucleic acids of the present invention can also be used to prime synthesis of nucleic acid, for purpose of either analysis or isolation, using mRNA, cDNA, or genomic DNA as template.

5 For use as primers, at least 17 contiguous nucleotides of the isolated nucleic acids of the present invention will be used. Often, at least 18, 19, or 20 contiguous nucleotides of the nucleic acids of the present invention will be used, and on occasion at least 20, 22, 24,
10 or 25 contiguous nucleotides of the nucleic acids of the present invention will be used, and even 30 nucleotides or more of the nucleic acids of the present invention can be used to prime specific synthesis.

The nucleic acid primers of the present invention
15 can be used, for example, to prime first strand cDNA synthesis on an mRNA template.

Such primer extension can be done directly to analyze the message. Alternatively, synthesis on an mRNA template can be done to produce first strand cDNA. The
20 first strand cDNA can thereafter be used, *inter alia*, directly as a single-stranded probe, as above-described, as a template for sequencing - permitting identification of alterations, including deletions, insertions, and substitutions, both normal allelic variants and mutations
25 associated with abnormal phenotypes- or as a template, either for second strand cDNA synthesis (e.g., as an antecedent to insertion into a cloning or expression vector), or for amplification.

The nucleic acid primers of the present invention
30 can also be used, for example, to prime single base extension (SBE) for SNP detection (see, e.g., U.S. Pat. No. 6,004,744, the disclosure of which is incorporated herein by reference in its entirety).

As another example, the nucleic acid primers of the present invention can be used to prime amplification of MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids, using transcript-derived or genomic DNA as template.

5 Primer-directed amplification methods are now well-established in the art. Methods for performing the polymerase chain reaction (PCR) are compiled, *inter alia*, in McPherson, PCR (Basics: From Background to Bench), Springer Verlag (2000) (ISBN: 0387916008); Innis et al. (eds.), PCR
10 Applications: Protocols for Functional Genomics, Academic Press (1999) (ISBN: 0123721857); Gelfand et al. (eds.), PCR Strategies, Academic Press (1998) (ISBN: 0123721822); Newton et al., PCR, Springer-Verlag New York (1997) (ISBN: 0387915060); Burke (ed.), PCR: Essential Techniques, John
15 Wiley & Son Ltd (1996) (ISBN: 047195697X); White (ed.), PCR Cloning Protocols: From Molecular Cloning to Genetic Engineering, Vol. 67, Humana Press (1996) (ISBN: 0896033430); McPherson et al. (eds.), PCR 2: A Practical Approach, Oxford University Press, Inc. (1995) (ISBN: 0199634254), the disclosures of which are incorporated
20 herein by reference in their entireties. Methods for performing RT-PCR are collected, e.g., in Siebert et al. (eds.), Gene Cloning and Analysis by RT-PCR, Eaton Publishing Company/Bio Techniques Books Division, 1998 (ISBN: 1881299147); Siebert (ed.), PCR Technique: RT-PCR, Eaton Publishing Company/BioTechniques Books (1995) (ISBN: 1881299139), the disclosure of which is incorporated
25 herein by reference in its entirety.

30 Isothermal amplification approaches, such as rolling circle amplification, are also now well-described. See, e.g., Schweitzer et al., *Curr. Opin. Biotechnol.* 12(1):21-7 (2001); U.S. Patent Nos. 6,235,502, 6,221,603, 6,210,884, 6,183,960, 5,854,033, 5,714,320, 5,648,245, and

international patent publications WO 97/19193 and
WO 00/15779, the disclosures of which are incorporated
herein by reference in their entireties. Rolling circle
amplification can be combined with other techniques to
5 facilitate SNP detection. See, e.g., Lizardi et al., *Nature*
Genet. 19(3):225-32 (1998).

As further described below, nucleic acids of the
present invention, inserted into vectors that flank the
nucleic acid insert with a phage promoter, such as T7, T3,
10 or SP6 promoter, can be used to drive *in vitro* expression of
RNA complementary to either strand of the nucleic acid of
the present invention. The RNA can be used, *inter alia*, as
a single-stranded probe, in cDNA-mRNA subtraction, or for *in*
vitro translation.

15 As will be further discussed herein below, nucleic
acids of the present invention that encode MDZ3, MDZ4, MDZ7
or MDZ12 protein or portions thereof can be used, *inter*
alia, to express the MDZ3, MDZ4, MDZ7 or MDZ12 proteins or
protein fragments, either alone, or as part of fusion
20 proteins.

Expression can be from genomic nucleic acids of
the present invention, or from transcript-derived nucleic
acids of the present invention.

Where protein expression is effected from genomic
25 DNA, expression will typically be effected in eukaryotic,
typically mammalian, cells capable of splicing introns from
the initial RNA transcript. Expression can be driven from
episomal vectors, such as EBV-based vectors, or can be
effected from genomic DNA integrated into a host cell
30 chromosome. As will be more fully described below, where
expression is from transcript-derived (or otherwise intron-
less) nucleic acids of the present invention, expression can

be effected in wide variety of prokaryotic or eukaryotic cells.

Expressed *in vitro*, the protein, protein fragment, or protein fusion can thereafter be isolated, to be used, *inter alia*, as a standard in immunoassays specific for the proteins, or protein isoforms, of the present invention; to be used as a therapeutic agent, e.g., to be administered as passive replacement therapy in individuals deficient in the proteins of the present invention, or to be administered as a vaccine; to be used for *in vitro* production of specific antibody, the antibody thereafter to be used, e.g., as an analytical reagent for detection and quantitation of the proteins of the present invention or to be used as an immunotherapeutic agent.

The isolated nucleic acids of the present invention can also be used to drive *in vivo* expression of the proteins of the present invention. *In vivo* expression can be driven from a vector - typically a viral vector, often a vector based upon a replication incompetent retrovirus, an adenovirus, or an adeno-associated virus (AAV) - for purpose of gene therapy. *In vivo* expression can also be driven from signals endogenous to the nucleic acid or from a vector, often a plasmid vector, such as pVAX1 (Invitrogen, Carlsbad CA, USA), for purpose of "naked" nucleic acid vaccination, as further described in U.S. Pat. Nos. 5,589,466; 5,679,647; 5,804,566; 5,830,877; 5,843,913; 5,880,104; 5,958,891; 5,985,847; 6,017,897; 6,110,898; 6,204,250, the disclosures of which are incorporated herein by reference in their entirety.

The nucleic acids of the present invention can also be used for antisense inhibition of transcription or translation. See Phillips (ed.), Antisense Technology, Part B, Methods in Enzymology Vol. 314, Academic Press, Inc.

- (1999) (ISBN: 012182215X); Phillips (ed.), Antisense Technology, Part A, Methods in Enzymology Vol. 313, Academic Press, Inc. (1999) (ISBN: 0121822141); Hartmann et al. (eds.), Manual of Antisense Methodology (Perspectives in Antisense Science), Kluwer Law International (1999) (ISBN: 079238539X); Stein et al. (eds.), Applied Antisense Oligonucleotide Technology, Wiley-Liss (cover (1998) (ISBN: 0471172790); Agrawal et al. (eds.), Antisense Research and Application, Springer-Verlag New York, Inc. (1998) (ISBN: 3540638334); Lichtenstein et al. (eds.), Antisense Technology: A Practical Approach, Vol. 185, Oxford University Press, INC. (1998) (ISBN: 0199635838); Gibson (ed.), Antisense and Ribozyme Methodology: Laboratory Companion, Chapman & Hall (1997) (ISBN: 3826100794); Chadwick et al. (eds.), Oligonucleotides as Therapeutic Agents - Symposium No. 209, John Wiley & Son Ltd (1997) (ISBN: 0471972797), the disclosures of which are incorporated herein by reference in their entireties.

Nucleic acids of the present invention, particularly cDNAs of the present invention, that encode full-length human MDZ3, MDZ4, MDZ7 or MDZ12 protein isoforms, have additional, well-recognized, immediate, real world utility as commercial products of manufacture suitable for sale.

For example, Invitrogen Corp. (Carlsbad, CA, USA), through its Research Genetics subsidiary, sells full length human cDNAs cloned into one of a selection of expression vectors as GeneStorm® expression-ready clones; utility is specific for the gene, since each gene is capable of being ordered separately and has a distinct catalogue number, and utility is substantial, each clone selling for \$650.00 US. Similarly, Incyte Genomics (Palo Alto, CA, USA) sells clones

from public and proprietary sources in multi-well plates or individual tubes.

Nucleic acids of the present invention that include genomic regions encoding the human MDZ3, MDZ4, MDZ7
5 or MDZ12 protein, or portions thereof, have yet further utilities.

For example, genomic nucleic acids of the present invention can be used as amplification substrates, e.g. for preparation of genome-derived single exon probes of the
10 present invention, as described above and in copending and commonly-owned U.S. patent application nos. 09/864,761, filed May 23, 2001, 09/774,203, filed January 29, 2001, and 09/632,366, filed August 3, 2000, the disclosures of which are incorporated herein by reference in their entireties.

As another example, genomic nucleic acids of the present invention can be integrated non-homologously into the genome of somatic cells, e.g. CHO cells, COS cells, or 293 cells, with or without amplification of the insertional locus, in order, e.g., to create stable cell lines capable
15 of producing the proteins of the present invention.

As another example, more fully described herein below, genomic nucleic acids of the present invention can be integrated nonhomologously into embryonic stem (ES) cells to create transgenic non-human animals capable of producing the
20 proteins of the present invention.

Genomic nucleic acids of the present invention can also be used to target homologous recombination to the human MDZ3, MDZ4, MDZ7 or MDZ12 locus, respectively. See, e.g., U.S. Patent Nos. 6,187,305; 6,204,061; 5,631,153; 5,627,059;
30 5,487,992; 5,464,764; 5,614,396; 5,527,695 and 6,063,630; and Kmiec et al. (eds.), Gene Targeting Protocols, Vol. 133, Humana Press (2000) (ISBN: 0896033600); Joyner (ed.), Gene Targeting: A Practical Approach, Oxford University Press,

Inc. (2000) (ISBN: 0199637938); Sedivy et al., Gene Targeting, Oxford University Press (1998) (ISBN: 071677013X); Tymms et al. (eds.), Gene Knockout Protocols, Humana Press (2000) (ISBN: 0896035727); Mak et al. (eds.), The Gene Knockout FactsBook, Vol. 2, Academic Press, Inc. (1998) (ISBN: 0124660444); Torres et al., Laboratory Protocols for Conditional Gene Targeting, Oxford University Press (1997) (ISBN: 019963677X); Vega (ed.), Gene Targeting, CRC Press, LLC (1994) (ISBN: 084938950X), the disclosures of which are incorporated herein by reference in their entireties.

Where the genomic region includes transcription regulatory elements, homologous recombination can be used to alter the expression of MDZ3, MDZ4, MDZ7 or MDZ12, both for purpose of *in vitro* production of MDZ3, MDZ4, MDZ7 or MDZ12 protein from human cells, and for purpose of gene therapy. See, e.g., U.S. Pat. Nos. 5,981,214, 6,048,524; 5,272,071.

Fragments of the nucleic acids of the present invention smaller than those typically used for homologous recombination can also be used for targeted gene correction or alteration, possibly by cellular mechanisms different from those engaged during homologous recombination.

For example, partially duplexed RNA/DNA chimeras have been shown to have utility in targeted gene correction, U.S. Pat. Nos. 5,945,339, 5,888,983, 5,871,984, 5,795,972, 5,780,296, 5,760,012, 5,756,325, 5,731,181, the disclosures of which are incorporated herein by reference in their entireties. So too have small oligonucleotides fused to triplexing domains have been shown to have utility in targeted gene correction, Culver et al., "Correction of chromosomal point mutations in human cells with bifunctional oligonucleotides," *Nature Biotechnol.* 17(10):989-93 (1999), as have oligonucleotides having modified terminal bases or

modified terminal internucleoside bonds, Gamper et al.,
Nucl. Acids Res. 28(21):4332-9 (2000), the disclosures of
which are incorporated herein by reference.

5 The isolated nucleic acids of the present
invention can also be used to provide the initial substrate
for recombinant engineering of MDZ3, MDZ4, MDZ7 or MDZ12
protein variants having desired phenotypic improvements.
Such engineering includes, for example, site-directed
mutagenesis, random mutagenesis with subsequent functional
10 screening, and more elegant schemes for recombinant
evolution of proteins, as are described, *inter alia*, in U.S.
Pat. Nos. 6,180,406; 6,165,793; 6,117,679; and 6,096,548,
the disclosures of which are incorporated herein by
reference in their entireties.

15 Nucleic acids of the present invention can be
obtained by using the labeled probes of the present
invention to probe nucleic acid samples, such as genomic
libraries, cDNA libraries, and mRNA samples, by standard
techniques. Nucleic acids of the present invention can also
20 be obtained by amplification, using the nucleic acid primers
of the present invention, as further demonstrated in Example
1, herein below. Nucleic acids of the present invention of
fewer than about 100 nt can also be synthesized chemically,
typically by solid phase synthesis using commercially
25 available automated synthesizers.

"Full Length" human MDZ3, MDZ4, MDZ7 or MDZ12
Nucleic Acids

30 In a first series of nucleic acid embodiments, the
invention provides isolated nucleic acids that encode the
entirety of the MDZ3, MDZ4, MDZ7 or MDZ12a, MDZ12bS, MDZ12bL
protein. As discussed above, the "full-length" nucleic

acids of the present invention can be used, *inter alia*, to express full length MDZ3, MDZ4, MDZ7 or MDZ12a, MDZ12bS, MDZ12bL protein. The full-length nucleic acids can also be used as nucleic acid probes; used as probes, the isolated
5 nucleic acids of these embodiments will respectively hybridize to MDZ3, MDZ4, MDZ7 or MDZ12, or to portions thereof.

In a first such embodiment, the invention provides an isolated nucleic acid comprising (i) the nucleotide
10 sequence of the nucleic acid of any of ATCC deposits _____ (MDZ3), _____ (MDZ4), _____ (MDZ7), and _____ (MDZ12a and MDZ12b), (ii) the nucleotide sequence of any of SEQ ID NOs: 1, 3027, 4407, 5770, 6938 or (iii) the complement of (i) or
15 (ii). The ATCC deposits have, and SEQ ID Nos: 1, 3027, 4407, 5770, 6938 present, the entire cDNA of MDZ3, MDZ4, MDZ7, and MDZ12a and MDZ12b, respectively, including the 5' untranslated (UT) region and 3' UT (except for MDZ12b).

In a second embodiment, the invention provides an
20 isolated nucleic acid comprising (i) the nucleotide sequence of SEQ ID Nos: 2, 3028, 4408, 5771, (ii) a degenerate variant of the nucleotide sequence of SEQ ID Nos: 2, 3028, 4408, 5771, or (iii) the complement of (i) or (ii). SEQ ID Nos: 2, 3028, 4408, 5771 present the open reading frame
25 (ORF) from SEQ ID Nos: 1, 3027, 4407, 5770, respectively.

In a third embodiment, the invention provides an isolated nucleic acid of no more than about 100 kb, often no more than about 75 kb, typically no more than about 50 kb, and usefully no more than about 25 kb, even no more than
30 about 10 kb, comprising (i) a nucleotide sequence that encodes a polypeptide with the amino acid sequence of any of SEQ ID Nos: 3, 3029, 4409, 5772, 6939, or 6940, or (ii) the complement of a nucleotide sequence that encodes a

polypeptide with the amino acid sequence of any of SEQ ID
Nos: 3, 3029, 4409, 5772, 6939, or 6940. SEQ ID Nos: 3,
3029, 4409, 5772, 6939, 6940 provide the amino acid sequence
of MDZ3, MDZ4, MDZ7, MDZ12a, MDZ12bS and MDZ12bL,
5 respectively.

In a fourth embodiment, the invention provides an
isolated nucleic acid of no more than about 100 kb, often no
more than about 75 kb, typically no more than about 50 kb,
and usefully no more than about 25 kb, even no more than
10 about 10 kb, having a nucleotide sequence that (i) encodes a
polypeptide having the sequence of any of SEQ ID NOS: 3,
3029, 4409, 5772, 6939, or 6940 with conservative amino acid
substitutions, (ii) encodes a polypeptide having the
sequence of any of SEQ ID Nos: 3, 3029, 4409, 5772, 6939, or
15 6940 with moderately conservative amino acid substitutions,
or (iii) the complement of (i) or (ii).

Selected Partial Nucleic Acids

20 In a second series of nucleic acid embodiments,
the invention provides isolated nucleic acids that encode
select portions of MDZ3, MDZ4, MDZ7 and MDZ12, respectively.

As will be further discussed herein below, these "partial"
nucleic acids can be used, *inter alia*, to express specific
25 portions of MDZ3, MDZ4, MDZ7 and MDZ12, respectively. These
"partial" nucleic acids can also be used, *inter alia*, as
nucleic probes.

Selected Partial Nucleic Acids of MDZ3

30 In a first such embodiment, the invention provides
an isolated nucleic acid comprising (i) the nucleotide
sequence of SEQ ID NO: 4, (ii) the nucleotide sequence of

SEQ ID NO:5, (iii) the nucleotide sequence of SEQ ID NO:6, (iv) a degenerate variant of SEQ ID NO:6, or (v) the complement of any of (i) - (iv). SEQ ID NO: 4 presents that portion of MDZ3 not present in known ESTs, with SEQ ID NO:5
5 representing the 5' UT portion of SEQ ID NO:4 and SEQ ID NO:6 representing the coding region of SEQ ID NO:4. Often, the isolated nucleic acids of this embodiment are no more than about 100 kb in length, often no more than about 75 kb in length, or 50 kb, or even 25 kb in length, and can be no
10 more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another embodiment, the invention provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes SEQ ID NO: 7 or (ii) the complement of a
15 nucleotide sequence that encodes SEQ ID NO: 7, wherein the isolated nucleic acid is no more than about 100 kb in length, often no more than about 75 kb in length, or 50 kb, or even 25 kb in length, and can be no more than about 15 kb in length, and frequently no more than about 10 kb in
20 length.

SEQ ID NO: 7 is the amino acid sequence encoded by SEQ ID NO:6.

In another embodiment, the invention provides an isolated nucleic acid comprising (i) a nucleotide sequence
25 that encodes SEQ ID NO: 7 with conservative substitutions, (ii) a nucleotide sequence that encodes SEQ ID NO: 7 with moderately conservative substitutions, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, often no more than
30 about 75 kb in length, or 50 kb, or even 25 kb in length, and can be no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a first such embodiment, the invention provides an isolated nucleic acid comprising (i) the nucleotide sequence of SEQ ID NO: 3030, (ii) the nucleotide sequence of SEQ ID NO: 3031, (iii) the nucleotide sequence of SEQ ID NO: 3032, (iv) a degenerate variant of the nucleotide sequence of SEQ ID NO: 3032, or (v) the complement of any of (i) - (iv). SEQ ID NO: 3030 presents a portion of MDZ4 not present in known ESTs, with SEQ ID NO:3031 representing the 5' UT portion of SEQ ID NO:3030 and SEQ ID NO:6 representing the coding region of SEQ ID NO:3030, wherein the isolated nucleic acids of this embodiment are no more than about 100 kb in length, often no more than about 75 kb in length, or 50 kb, or even 25 kb in length, and can be no more than about 15 kb in length, and frequently no more than about 10 kb in length.

30 In another embodiment, the invention provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes SEQ ID NO: 3033 with conservative substitutions, (ii) a nucleotide sequence that encodes SEQ

ID NO:3033 with moderately conservative substitutions, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another embodiment, the invention provides an isolated nucleic acid comprising (i) the nucleotide sequence of SEQ ID NO: 3034, (ii) the nucleotide sequence of SEQ ID NO: 3035, (iii) a degenerate variant of the nucleotide sequence of SEQ ID NO:3035, (iv) the nucleotide sequence of SEQ ID NO:3036, or (v) the complement of any of (i) - (iv), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

SEQ ID NO: 3034 presents a portion of MDZ4 not present in known ESTs, with SEQ ID NO:3035 representing a coding region portion of SEQ ID NO:3034, and with SEQ ID NO:3036 representing the 3' UT portion of SEQ ID NO:3034.

In another embodiment, the invention provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes SEQ ID NO: 3037 or (ii) the complement of a nucleotide sequence that encodes SEQ ID NO: 3037, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, frequently no more than about 50 kb in length. SEQ ID NO: 3037 is the amino acid sequence encoded by SEQ ID NO:3035. Often, the isolated nucleic acids of this embodiment are no

more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another embodiment, the invention provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes SEQ ID NO: 3037 with conservative substitutions, (ii) a nucleotide sequence that encodes SEQ ID NO: 3037 with moderately conservative substitutions, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

Selected Partial Nucleic Acids of MDZ7

In a first such embodiment, the invention provides an isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO: 4410 or SEQ ID NO:4411, or the complement thereof, wherein the isolated nucleic acid of this aspect of the invention is no more than about 100 kb in length, often no more than about 75 kb in length, more typically no more than about 50 kb length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

SEQ ID Nos: 4410 and 4411 present those portions of MDZ7 not present in known ESTs.

Selected Partial Nucleic Acids of MDZ12

In a first such embodiment, the invention provides an isolated nucleic acid comprising (i) the nucleotide
5 sequence of SEQ ID NO: 5773, (ii) a degenerate variant of
SEQ ID NO: 5773, or (iii) the complement of (i) or (ii),
wherein the isolated nucleic acid is no more than about 100
kb in length, typically no more than about 75 kb in length,
more typically no more than about 50 kb length. Often, the
10 isolated nucleic acids of this embodiment are no more than
about 25 kb in length, often no more than about 15 kb in
length, and frequently no more than about 10 kb in length.

SEQ ID NO: 5773 encodes a portion of MDZ12a not
present in known ESTs.

15 In another embodiment, the invention provides an
isolated nucleic acid comprising (i) a nucleotide sequence
that encodes SEQ ID NO: 5774 or (ii) the complement of a
nucleotide sequence that encodes SEQ ID NO: 5774, wherein
the isolated nucleic acid is no more than about 100 kb in
20 length, typically no more than about 75 kb in length,
frequently no more than about 50 kb in length. SEQ ID NO:
5774 is the amino acid sequence encoded by that portion of
MDZ12a not found in any EST fragments. Often, the isolated
nucleic acids of this embodiment are no more than about 25
25 kb in length, often no more than about 15 kb in length, and
frequently no more than about 10 kb in length.

In another embodiment, the invention provides an
isolated nucleic acid comprising (i) a nucleotide sequence
that encodes SEQ ID NO: 5774 with conservative
30 substitutions, (ii) a nucleotide sequence that encodes SEQ
ID NO: 5774 with moderately conservative substitutions, or
(iii) the complement of (i) or (ii), wherein the isolated
nucleic acid is no more than about 100 kb in length,

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typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another such embodiment, the invention provides an isolated nucleic acid comprising (i) the nucleotide sequence of SEQ ID NO: 6941, (ii) a degenerate variant of SEQ ID NO: 6941, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb length. SEQ ID NO: 6941 is the novel exon inserted in the MDZ12b splice variant. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another embodiment, the invention provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes SEQ ID NO: 6942 or (ii) the complement of a nucleotide sequence that encodes SEQ ID NO: 6942, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, frequently no more than about 50 kb in length. SEQ ID NO: 6942 is the amino acid sequence encoded by the novel MDZ12b exon before the stop codon. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another embodiment, the invention provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes SEQ ID NO: 6942 with conservative substitutions, (ii) a nucleotide sequence that encodes SEQ

ID NO: 6942 with moderately conservative substitutions, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

10 Cross-Hybridizing Nucleic Acids

 In another series of nucleic acid embodiments, the invention provides isolated nucleic acids that hybridize to various of the MDZ3, MDZ4, MDZ7 and MDZ12 nucleic acids of the present invention. These cross-hybridizing nucleic acids can be used, *inter alia*, as probes for, and to drive expression of, proteins that are related to MDZ3, MDZ4, MDZ7 and MDZ12 of the present invention as further isoforms, homologues, paralogues, or orthologues.

20 Cross-Hybridizing Nucleic Acids of MDZ3

 In a first such embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a probe the nucleotide sequence of which consists of at least 17 nt, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, or 50 nt of SEQ ID NO: 4 or of the complement of SEQ ID NO: 4, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25

kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under moderate stringency conditions to a probe the nucleotide sequence of which consists of at least 17 nt, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, or 50 nt of SEQ ID NO: 4 or of the complement of SEQ ID NO: 4, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a hybridization probe the nucleotide sequence of which (i) encodes a polypeptide having the sequence of SEQ ID NO: 7, (ii) encodes a polypeptide having the sequence of SEQ ID NO: 7 with conservative amino acid substitutions, or (iii) is the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

Cross-Hybridizing Nucleic Acids of MDZ4

In a first such embodiment, the invention provides an isolated nucleic acid comprising a sequence that

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embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In yet another embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a probe the nucleotide sequence of which consists of at least 17 nt, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, or 50 nt of SEQ ID NO: 3034 or of the complement of SEQ ID NO: 3034, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under moderate stringency conditions to a probe the nucleotide sequence of which consists of at least 17 nt, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, or 50 nt of SEQ ID NO: 3034 or of the complement of SEQ ID NO: 3034, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a hybridization probe the nucleotide sequence of which (i) encodes a polypeptide having the sequence of SEQ ID NO: 3037, (ii) encodes a polypeptide having the sequence of SEQ ID NO: 3037 with

conservative amino acid substitutions, or (iii) is the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

10 Cross-Hybridizing Nucleic Acids of MDZ7

 In a first such embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a probe the nucleotide sequence of which consists of SEQ ID NO: 4410 or the complement of SEQ ID NO: 4410, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

 In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under moderate stringency conditions to a probe the nucleotide sequence of which consists of SEQ ID NO: 4410 or the complement of SEQ ID NO: 4410, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a probe the nucleotide sequence of which consists of SEQ ID NO: 4411 or the
5 complement of SEQ ID NO: 4411, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length,
10 often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under moderate stringency conditions to a probe the
15 nucleotide sequence of which consists of SEQ ID NO: 4411 or the complement of SEQ ID NO: 4411, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated
20 nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

Cross-Hybridizing Nucleic Acids of MDZ12

25 In a first such embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a probe the nucleotide sequence of which consists of SEQ ID NO: 5773 or
30 the complement of SEQ ID NO: 5773, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated

nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under moderate stringency conditions to a probe the nucleotide sequence of which consists of SEQ ID NO: 5773 or the complement of SEQ ID NO: 5773, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a hybridization probe the nucleotide sequence of which (i) encodes a polypeptide having the sequence of SEQ ID NO: 5774, (ii) encodes a polypeptide having the sequence of SEQ ID NO: 5774 with conservative amino acid substitutions, or (iii) is the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, and often no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another such embodiment, the invention provides an isolated nucleic acid comprising a sequence that hybridizes under high stringency conditions to a probe the nucleotide sequence of which consists of SEQ ID NO: 6941 or the complement of SEQ ID NO: 6941, wherein the isolated

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Particularly Useful Nucleic Acids of MDZ3

Also particularly useful among the above-described MDZ3 nucleic acids are those that encode, or the complement of which encode, a polypeptide having at least one C2H2 (Kruppel family) zinc finger, and especially those that encode 7 C2H2 zinc fingers in tandem, those that encode a SCAN domain, those that encode a KRAB domain, and those that include all of a SCAN domain, KRAB domain, and 7 zinc fingers.

Particularly Useful Nucleic Acids of MDZ4

30 Other particularly useful embodiments of the MDZ4
nucleic acids above-described are those that encode, or the
complement of which encode, a polypeptide that has at least
one C2H2 (Kruppel family) zinc finger, and especially those

that encode 5 C2H2 zinc fingers in tandem, those that encode a SCAN domain, and those that include all of a SCAN domain and 5 zinc fingers.

Also particularly useful among the above-described MDZ4 nucleic acids are those that encode, or the complement of which encode, a polypeptide having sequence-specific nucleic acid binding regulatory activity, and that participates in protein-protein interactions with other transcription modulators.

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Particularly Useful Nucleic Acids of MDZ7

Particularly useful among the above-described MDZ7 nucleic acids are those that are expressed, or the complement of which are expressed, in testes, preferably at a level greater than that in kidney, liver, lung, brain or heart, typically at a level at least two-fold that in kidney, liver, lung, brain or heart, often at least three-fold, four-fold, or even five-fold that in kidney, liver, lung, brain or heart.

Also particularly useful among the above-described MDZ7 nucleic acids are those that encode, or the complement of which encode, a polypeptide having at least one C2H2 (Kruppel family) zinc finger, especially those having a plurality of zinc fingers in tandem, particularly those having 7 zinc fingers in tandem.

Also particularly useful among the above-described MDZ7 nucleic acids are those that encode, or the complement of which encode, a polypeptide having sequence-specific nucleic acid binding regulatory activity, and that functions in sequence-specific modulation of gene expression.

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Particularly Useful Nucleic Acids of MDZ12

Particularly useful among the above-described MDZ12 nucleic acids are those that are expressed, or the complement of which are expressed, in brain, heart, kidney, placenta, skeletal muscle, testis, Hela cells, bone marrow and liver.

Also particularly useful among the above-described nucleic acids are those that encode, or the complement of which encode, a polypeptide having a C2H2 (Kruppel family) zinc finger, particularly those having a plurality of such zinc fingers in tandem, especially those having at least 5, often at least 12, zinc fingers in tandem. Also particularly useful among the above-described nucleic acids are those that encode, or the complement of which encode, a polypeptide having KRAB-B domain, especially those having both a KRAB domain and at least one, preferably a plurality, especially at least 10, often at least 12, zinc finger domains.

Particularly useful nucleic acids are those that encode, or the complement of which encode, polypeptides that act as sequence-specific transcription regulators, and that interaction with other transcriptional modulators by protein-protein interactions.

Nucleic Acid Fragments

In another series of nucleic acid embodiments, the invention provides fragments of various of the isolated nucleic acids of the present invention which prove useful, *inter alia*, as nucleic acid probes, as amplification primers, and to direct expression or synthesis of epitopic or immunogenic protein fragments.

Nucleic Acid Fragments of MDZ3

In a first embodiment, the invention provides an isolated nucleic acid comprising at least 17 nucleotides, 18
5 nucleotides, 20 nucleotides, 24 nucleotides, or 25
nucleotides of (i) SEQ ID NO: 4, (ii) a degenerate variant
of SEQ ID NO: 6, or (iii) the complement of (i) or (ii),
wherein the isolated nucleic acid is no more than about 100
kb in length, typically no more than about 75 kb in length,
10 more typically no more than about 50 kb in length. Often,
the isolated nucleic acids of this embodiment are no more
than about 25 kb in length, often no more than about 15 kb
in length, and frequently no more than about 10 kb in
length.

15 The invention also provides an isolated nucleic
acid comprising (i) a nucleotide sequence that encodes a
peptide of at least 8 contiguous amino acids of SEQ ID NO:
7, or (ii) the complement of a nucleotide sequence that
encodes a peptide of at least 8 contiguous amino acids of
20 SEQ ID NO: 7, wherein the isolated nucleic acid is no more
than about 100 kb in length, typically no more than about 75
kb in length, more typically no more than about 50 kb in
length. Often, the isolated nucleic acids of this
embodiment are no more than about 25 kb in length, often no
25 more than about 15 kb in length, and frequently no more than
about 10 kb in length.

The invention also provides an isolated nucleic
acid comprising (i) a nucleotide sequence that encodes a
peptide of at least 15 contiguous amino acids of SEQ ID NO:
30 7, or (ii) the complement of a nucleotide sequence that
encodes a peptide of at least 15 contiguous amino acids of
SEQ ID NO: 7, wherein the isolated nucleic acid is no more
than about 100 kb in length, typically no more than about 75

kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising a nucleotide sequence that encodes (i) a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO: 7 with conservative amino acid substitutions, (ii) a polypeptide having the sequence of at least 15 contiguous amino acids of SEQ ID NO:7 with conservative amino acid substitutions, (iii) a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO:7 with moderately conservative substitutions, (iv) a polypeptide having the sequence of at last 15 congruous amino acids of SEQ ID NO:7 with moderately conservative substitutions, or (v) the complement of any of (i) - (iv), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

Nucleic Acid Fragments of MDZ4

In a first embodiment, the invention provides an isolated nucleic acid comprising at least 17 nucleotides, 18 nucleotides, 20 nucleotides, 24 nucleotides, or 25 nucleotides of (i) SEQ ID NO: 3030, (ii) a degenerate variant of SEQ ID NO: 3032, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than

about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes a peptide of at least 8 contiguous amino acids of SEQ ID NO: 3033, (ii) a nucleotide sequence that encodes a peptide of at least 15 contiguous amino acids of SEQ ID NO:3033, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising a nucleotide sequence that (i) encodes a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO: 3033 with conservative amino acid substitutions, (ii) encodes a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO: 3033 with moderately conservative amino acid substitutions, (iii) encodes a polypeptide having the sequence of at least 15 contiguous amino acids of SEQ ID NO:3033 with conservative amino acid substitutions, (iv) encodes a polypeptide having the sequence of at least 15 contiguous amino acids of sEQ ID NO:3033 with moderately conservative amino acid substitutions, or (v) the complement of any one of (i) - (iv), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75

kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a further embodiment, the invention provides an isolated nucleic acid comprising at least 17 nucleotides, 18 nucleotides, 20 nucleotides, 24 nucleotides, or 25 nucleotides of (i) SEQ ID NO: 3034, (ii) a degenerate variant of SEQ ID NO: 3035, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes a peptide of at least 8 contiguous amino acids of SEQ ID NO: 3037, or (ii) the complement of a nucleotide sequence that encodes a peptide of at least 8 contiguous amino acids of SEQ ID NO: 3037, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising a nucleotide sequence that (i) encodes a polypeptide having the sequence of at least 8 contiguous

amino acids of SEQ ID NO: 3037 having conservative amino acid substitutions, (ii) encodes a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO: 3037 having moderately conservative amino acid substitutions, (iii) encodes a polypeptide having the sequence of at least 15 contiguous amino acids of SEQ ID NO: 3037 having conservative amino acid substitutions, (iv) encodes a polypeptide having the sequence of at least 15 contiguous amino acids of SEQ ID NO: 3037 having moderately conservative amino acid substitutions, or (v) is the complement of any of (i) - (iv), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

Nucleic Acid Fragments of MDZ7

In a first embodiment of this aspect of the invention, the invention provides an isolated nucleic acid comprising at least 17 nucleotides, 18 nucleotides, 20 nucleotides, 24 nucleotides, or 25 nucleotides of (i) SEQ ID NO: 4410, or (ii) the complement thereof, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In a second embodiment, the invention provides an isolated nucleic acid comprising at least 17 nucleotides, 18 nucleotides, 20 nucleotides, 24 nucleotides, or 25 nucleotides of (i) SEQ ID NO: 4411, or (ii) the complement of thereof, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

Nucleic Acid Fragments of MDZ12

In a first embodiment, the invention provides an isolated nucleic acid comprising at least 17 nucleotides, 18 nucleotides, 20 nucleotides, 24 nucleotides, or 25 nucleotides of (i) SEQ ID NO: 5773, (ii) a degenerate variant of SEQ ID NO: 5773, or (iii) the complement of (i) or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes a peptide of at least 8 contiguous amino acids of SEQ ID NO: 5774, or (ii) the complement of a nucleotide sequence that encodes a peptide of at least 8 contiguous amino acids of SEQ ID NO: 5774, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than

about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes a peptide of at least 15 contiguous amino acids of SEQ ID NO: 5774, or (ii) the complement of a nucleotide sequence that encodes a peptide of at least 15 contiguous amino acids of SEQ ID NO: 5774, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising a nucleotide sequence that (i) encodes a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO: 5774 with conservative amino acid substitutions, or (ii) encodes a polypeptide having the sequence of at least 15 contiguous amino acids of SEQ ID NO: 5774 having conservative amino acid substitutions, (iii) encodes a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO: 5774 with moderately conservative amino acid substitutions, (iv) encodes a polypeptide having the sequence of at least 15 contiguous amino acids of SEQ ID NO: 5774 having moderately conservative amino acid substitutions, or (v) the complement of any one of (i) - (iv), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in

length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

5 In another embodiment, the invention provides an isolated nucleic acid comprising at least 17 nucleotides, 18 nucleotides, 20 nucleotides, 24 nucleotides, or 25 nucleotides of (i) SEQ ID NO: 6941, (ii) a degenerate variant of SEQ ID NO: 6941, or (iii) the complement of (i)
10 or (ii), wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no
15 more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic acid comprising (i) a nucleotide sequence that encodes a peptide of at least 8 contiguous amino acids of SEQ ID NO:
20 6942, or (ii) the complement of a nucleotide sequence that encodes a peptide of at least 8 contiguous amino acids of SEQ ID NO: 6942, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50
25 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

The invention also provides an isolated nucleic
30 acid comprising a nucleotide sequence that (i) encodes a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO: 6942 with conservative amino acid substitutions, (ii) encodes a polypeptide having the

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sequence of at least 15 contiguous amino acids of SEQ ID NO: 6942 with conservative amino acid substitutions, (iii) encodes a polypeptide having the sequence of at least 8 contiguous amino acids of SEQ ID NO: 6942 with moderately conservative amino acid substitutions, (iv) encodes a polypeptide having the sequence of at least 15 contiguous amino acids of SEQ ID NO: 6942 with moderately conservative amino acid substitutions, or (v) the complement of any one of (i) - (iv).

Single Exon Probes

The invention further provides genome-derived single exon probes having portions of no more than one exon of the above-described genes. As further described in commonly owned and copending U.S. patent application serial no. 09/632,366, filed August 3, 2000 ("Methods and Apparatus for High Throughput Detection and Characterization of alternatively Spliced Genes"), the disclosure of which is incorporated herein by reference in its entirety, such single exon probes have particular utility in identifying and characterizing splice variants. In particular, such single exon probes are useful for identifying and discriminating the expression of distinct isoforms of genes.

Single Exon Probes of MDZ3

The invention further provides genome-derived single exon probes having portions of no more than one exon of the MDZ3 gene.

In a first embodiment, the invention provides an isolated nucleic acid comprising a nucleotide sequence of no more than one portion of SEQ ID NOs: 8 - 15 or the

complement of SEQ ID NOs: 8 - 15, wherein the portion comprises at least 17 contiguous nucleotides, 18 contiguous nucleotides, 20 contiguous nucleotides, 24 contiguous nucleotides, 25 contiguous nucleotides, or 50 contiguous nucleotides of any one of SEQ ID Nos: 8 - 15, or their complement. In a further embodiment, the exonic portion comprises the entirety of the referenced SEQ ID NO: or its complement.

In other embodiments, the invention provides isolated single exon probes having the nucleotide sequence of any one of SEQ ID NOs: 16 - 23.

Single Exon Probes of MDZ4

The invention further provides genome-derived single exon probes having portions of no more than one exon of the MDZ4 gene.

In a first embodiment, the invention provides an isolated nucleic acid comprising a nucleotide sequence of no more than one portion of SEQ ID NOs: 3038 - 3041 or the complement of SEQ ID NOs: 3038 - 3041, wherein the portion comprises at least 17 contiguous nucleotides, 18 contiguous nucleotides, 20 contiguous nucleotides, 24 contiguous nucleotides, 25 contiguous nucleotides, or 50 contiguous nucleotides of any one of SEQ ID NOs: 3038 - 3041, or their complement. In a further embodiment, the exonic portion comprises the entirety of the referenced SEQ ID NO: or its complement.

In other embodiments, the invention provides isolated single exon probes having the nucleotide sequence of any one of SEQ ID NOs: 3042 - 3045.

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Single Exon Probes of MDZ7

The invention further provides genome-derived single exon probes having portions of no more than one exon
5 of the MDZ7 gene.

In a first embodiment, the invention provides an isolated nucleic acid comprising a nucleotide sequence of no more than one portion of SEQ ID NOs: 4412 - 4415 or the complement of SEQ ID NOs: 4412 - 4415, wherein the portion
10 comprises at least 17 contiguous nucleotides, 18 contiguous nucleotides, 20 contiguous nucleotides, 24 contiguous nucleotides, 25 contiguous nucleotides, or 50 contiguous nucleotides of any one of SEQ ID NOs: 4412 - 4415, or their complement. In a further embodiment, the exonic portion
15 comprises the entirety of the referenced SEQ ID NO: or its complement.

In other embodiments, the invention provides isolated single exon probes having the nucleotide sequence of any one of SEQ ID NOs: 4416 - 4419.

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Single Exon Probes of MDZ12

The invention further provides genome-derived single exon probes having portions of no more than one exon
25 of the MDZ12 gene.

In a first embodiment, the invention provides an isolated nucleic acid comprising a nucleotide sequence of no more than one portion of SEQ ID NOs: 5775 - 5778, 6941 or the complement of SEQ ID NOs: 5775 - 5778, 6941 wherein the
30 portion comprises at least 17 contiguous nucleotides, 18 contiguous nucleotides, 20 contiguous nucleotides, 24 contiguous nucleotides, 25 contiguous nucleotides, or 50 contiguous nucleotides of any one of SEQ ID NOs: 5775 -

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5778, 6941, or their complement. In a further embodiment, the exonic portion comprises the entirety of the referenced SEQ ID NO: or its complement.

In other embodiments, the invention provides
5 isolated single exon probes having the nucleotide sequence of any one of SEQ ID NOs: 5779 -5782 and 6941.

Transcription Control Nucleic Acids

10 Transcription Control Nucleic Acids of MDZ3

In another aspect, the present invention provides genome-derived isolated nucleic acids that include nucleic acid sequence elements that control transcription of the
15 MDZ3 gene. These nucleic acids can be used, *inter alia*, to drive expression of heterologous coding regions in recombinant constructs, thus conferring upon such heterologous coding regions the expression pattern of the native MDZ3 gene. These nucleic acids can also be used,
20 conversely, to target heterologous transcription control elements to the MDZ3 genomic locus, altering the expression pattern of the MDZ3 gene itself.

In a first such embodiment, the invention provides an isolated nucleic acid comprising the nucleotide sequence
25 of SEQ ID NO: 24 or its complement, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25
30 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another embodiment, the invention provides an isolated nucleic acid comprising at least 17, 18, 20, 24, or

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25 nucleotides of the sequence of SEQ ID NO: 3046 or its complement, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

10 Transcription Control Nucleic Acids of MDZ7

 In another aspect, the present invention provides genome-derived isolated nucleic acids that include nucleic acid sequence elements that control transcription of the MDZ7 gene. These nucleic acids can be used, *inter alia*, to drive expression of heterologous coding regions in recombinant constructs, thus conferring upon such heterologous coding regions the expression pattern of the native MDZ7 gene. These nucleic acids can also be used, conversely, to target heterologous transcription control elements to the MDZ7 genomic locus, altering the expression pattern of the MDZ7 gene itself.

 In a first such embodiment, the invention provides an isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO: 4420 or its complement, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

 In another embodiment, the invention provides an isolated nucleic acid comprising at least 17, 18, 20, 24, or

25 nucleotides of the sequence of SEQ ID NO: 4420 or its complement, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

10 Transcription Control Nucleic Acids of MDZ12

In another aspect, the present invention provides genome-derived isolated nucleic acids that include nucleic acid sequence elements that control transcription of the MDZ12 gene. These nucleic acids can be used, *inter alia*, to drive expression of heterologous coding regions in recombinant constructs, thus conferring upon such heterologous coding regions the expression pattern of the native MDZ12 gene. These nucleic acids can also be used, conversely, to target heterologous transcription control elements to the MDZ12 genomic locus, altering the expression pattern of the MDZ12 gene itself.

In a first such embodiment, the invention provides an isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO: 5783 or its complement, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

In another embodiment, the invention provides an isolated nucleic acid comprising at least 17, 18, 20, 24, or

25 nucleotides of the sequence of SEQ ID NO: 5783 or its complement, wherein the isolated nucleic acid is no more than about 100 kb in length, typically no more than about 75 kb in length, more typically no more than about 50 kb in length. Often, the isolated nucleic acids of this embodiment are no more than about 25 kb in length, often no more than about 15 kb in length, and frequently no more than about 10 kb in length.

10 VECTORS AND HOST CELLS

In another aspect, the present invention provides vectors that comprise one or more of the isolated nucleic acids of the present invention, and host cells in which such vectors have been introduced.

The vectors can be used, *inter alia*, for propagating the nucleic acids of the present invention in host cells (cloning vectors), for shuttling the nucleic acids of the present invention between host cells derived from disparate organisms (shuttle vectors), for inserting the nucleic acids of the present invention into host cell chromosomes (insertion vectors), for expressing sense or antisense RNA transcripts of the nucleic acids of the present invention *in vitro* or within a host cell, and for expressing polypeptides encoded by the nucleic acids of the present invention, alone or as fusions to heterologous polypeptides. Vectors of the present invention will often be suitable for several such uses.

Vectors are by now well-known in the art, and are described, *inter alia*, in Jones et al. (eds.), Vectors: Cloning Applications : Essential Techniques (Essential Techniques Series), John Wiley & Son Ltd 1998 (ISBN: 047196266X); Jones et al. (eds.), Vectors: Expression

Systems : Essential Techniques (Essential Techniques Series), John Wiley & Son Ltd, 1998 (ISBN:0471962678); Gacesa et al., Vectors: Essential Data, John Wiley & Sons, 1995 (ISBN: 0471948411); Cid-Arregui (eds.), Viral Vectors: Basic Science and Gene Therapy, Eaton Publishing Co., 2000 (ISBN: 188129935X); Sambrook et al., Molecular Cloning: A Laboratory Manual (3rd ed.), Cold Spring Harbor Laboratory Press, 2001 (ISBN: 0879695773); Ausubel et al. (eds.), Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology (4th ed.), John Wiley & Sons, 1999 (ISBN: 047132938X), the disclosures of which are incorporated herein by reference in their entireties. Furthermore, an enormous variety of vectors are available commercially. Use of existing vectors and modifications thereof being well within the skill in the art, only basic features need be described here.

Typically, vectors are derived from virus, plasmid, prokaryotic or eukaryotic chromosomal elements, or some combination thereof, and include at least one origin of replication, at least one site for insertion of heterologous nucleic acid, typically in the form of a polylinker with multiple, tightly clustered, single cutting restriction sites, and at least one selectable marker, although some integrative vectors will lack an origin that is functional in the host to be chromosomally modified, and some vectors will lack selectable markers. Vectors of the present invention will further include at least one nucleic acid of the present invention inserted into the vector in at least one location.

Where present, the origin of replication and selectable markers are chosen based upon the desired host cell or host cells; the host cells, in turn, are selected based upon the desired application.

For example, prokaryotic cells, typically *E. coli*, are typically chosen for cloning. In such case, vector replication is predicated on the replication strategies of coliform-infecting phage - such as phage lambda, M13, T7, T3 and P1 - or on the replication origin of autonomously replicating episomes, notably the ColE1 plasmid and later derivatives, including pBR322 and the pUC series plasmids. Where *E. coli* is used as host, selectable markers are, analogously, chosen for selectivity in gram negative bacteria: e.g., typical markers confer resistance to antibiotics, such as ampicillin, tetracycline, chloramphenicol, kanamycin, streptomycin, zeocin; auxotrophic markers can also be used.

As another example, yeast cells, typically *S. cerevisiae*, are chosen, *inter alia*, for eukaryotic genetic studies, due to the ease of targeting genetic changes by homologous recombination and to the ready ability to complement genetic defects using recombinantly expressed proteins, for identification of interacting protein components, e.g. through use of a two-hybrid system, and for protein expression. Vectors of the present invention for use in yeast will typically, but not invariably, contain an origin of replication suitable for use in yeast and a selectable marker that is functional in yeast.

Integrative YIp vectors do not replicate autonomously, but integrate, typically in single copy, into the yeast genome at low frequencies and thus replicate as part of the host cell chromosome; these vectors lack an origin of replication that is functional in yeast, although they typically have at least one origin of replication suitable for propagation of the vector in bacterial cells. YE_p vectors, in contrast, replicate episomally and autonomously due to presence of the yeast 2 micron plasmid

origin (2 μ m ori). The YCp yeast centromere plasmid vectors are autonomously replicating vectors containing centromere sequences, CEN, and autonomously replicating sequences, ARS; the ARS sequences are believed to correspond to the natural
5 replication origins of yeast chromosomes. YACs are based on yeast linear plasmids, denoted YLp, containing homologous or heterologous DNA sequences that function as telomeres (TEL) *in vivo*, as well as containing yeast ARS (origins of replication) and CEN (centromeres) segments.

10 Selectable markers in yeast vectors include a variety of auxotrophic markers, the most common of which are (in *Saccharomyces cerevisiae*) URA3, HIS3, LEU2, TRP1 and LYS2, which complement specific auxotrophic mutations, such as ura3-52, his3-D1, leu2-D1, trp1-D1 and lys2-201. The
15 URA3 and LYS2 yeast genes further permit negative selection based on specific inhibitors, 5-fluoro-orotic acid (FOA) and α -aminoadipic acid (α AA), respectively, that prevent growth of the prototrophic strains but allows growth of the ura3 and lys2 mutants, respectively. Other selectable markers
20 confer resistance to, e.g., zeocin.

As yet another example, insect cells are often chosen for high efficiency protein expression. Where the host cells are from *Spodoptera frugiperda* - e.g., Sf9 and Sf21 cell lines, and expresSFTM cells (Protein Sciences
25 Corp., Meriden, CT, USA) - the vector replicative strategy is typically based upon the baculovirus life cycle. Typically, baculovirus transfer vectors are used to replace the wild-type AcMNPV polyhedrin gene with a heterologous gene of interest. Sequences that flank the polyhedrin gene
30 in the wild-type genome are positioned 5' and 3' of the expression cassette on the transfer vectors. Following cotransfection with AcMNPV DNA, a homologous recombination event occurs between these sequences resulting in a

recombinant virus carrying the gene of interest and the polyhedrin or p10 promoter. Selection can be based upon visual screening for lacZ fusion activity.

As yet another example, mammalian cells are often
5 chosen for expression of proteins intended as pharmaceutical agents, and are also chosen as host cells for screening of potential agonist and antagonists of a protein or a physiological pathway.

Where mammalian cells are chosen as host cells,
10 vectors intended for autonomous extrachromosomal replication will typically include a viral origin, such as the SV40 origin (for replication in cell lines expressing the large T-antigen, such as COS1 and COS7 cells), the papillomavirus origin, or the EBV origin for long term episomal replication
15 (for use, e.g., in 293-EBNA cells, which constitutively express the EBV EBNA-1 gene product and adenovirus E1A). Vectors intended for integration, and thus replication as part of the mammalian chromosome, can, but need not, include an origin of replication functional in mammalian cells, such
20 as the SV40 origin. Vectors based upon viruses, such as adenovirus, adeno-associated virus, vaccinia virus, and various mammalian retroviruses, will typically replicate according to the viral replicative strategy.

Selectable markers for use in mammalian cells
25 include resistance to neomycin (G418), blasticidin, hygromycin and to zeocin, and selection based upon the purine salvage pathway using HAT medium.

Plant cells can also be used for expression, with the vector replicon typically derived from a plant virus
30 (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) and selectable markers chosen for suitability in plants.

For propagation of nucleic acids of the present invention that are larger than can readily be accommodated in vectors derived from plasmids or virus, the invention further provides artificial chromosomes - BACs, YACs, HACs and PACs - that comprise MDZ3, MDZ4, MDZ7 and MDZ12 nucleic acids, respectively, often genomic nucleic acids.

The BAC system is based on the well-characterized *E. coli* F-factor, a low copy plasmid that exists in a supercoiled circular form in host cells. The structural features of the F-factor allow stable maintenance of individual human DNA clones as well as easy manipulation of the cloned DNA. See Shizuya *et al.*, *Keio J. Med.* 50(1):26-30 (2001); Shizuya *et al.*, *Proc. Natl. Acad. Sci. USA* 89(18):8794-7 (1992).

YACs are based on yeast linear plasmids, denoted YLp, containing homologous or heterologous DNA sequences that function as telomeres (TEL) *in vivo*, as well as containing yeast ARS (origins of replication) and CEN (centromeres) segments.

HACs are human artificial chromosomes. Kuroiwa *et al.*, *Nature Biotechnol.* 18(10):1086-90 (2000); Henning *et al.*, *Proc. Natl. Acad. Sci. USA* 96(2):592-7 (1999); Harrington *et al.*, *Nature Genet.* 15(4):345-55 (1997). In one version, long synthetic arrays of alpha satellite DNA are combined with telomeric DNA and genomic DNA to generate linear microchromosomes that are mitotically and cytogenetically stable in the absence of selection.

PACs are P1-derived artificial chromosomes. Sternberg, *Proc. Natl. Acad. Sci. USA* 87(1):103-7 (1990); Sternberg *et al.*, *New Biol.* 2(2):151-62 (1990); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 89(6):2056-60 (1992).

Vectors of the present invention will also often include elements that permit *in vitro* transcription of RNA

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from the inserted heterologous nucleic acid. Such vectors typically include a phage promoter, such as that from T7, T3, or SP6, flanking the nucleic acid insert. Often two different such promoters flank the inserted nucleic acid, 5 permitting separate *in vitro* production of both sense and antisense strands.

Expression vectors of the present invention - that is, those vectors that will drive expression of polypeptides from the inserted heterologous nucleic acid - will often 10 include a variety of other genetic elements operatively linked to the protein-encoding heterologous nucleic acid insert, typically genetic elements that drive transcription, such as promoters and enhancer elements, those that facilitate RNA processing, such as transcription termination 15 and/or polyadenylation signals, and those that facilitate translation, such as ribosomal consensus sequences.

For example, vectors for expressing proteins of the present invention in prokaryotic cells, typically *E. coli*, will include a promoter, often a phage promoter, such 20 as phage lambda pL promoter, the trc promoter, a hybrid derived from the trp and lac promoters, the bacteriophage T7 promoter (in *E. coli* cells engineered to express the T7 polymerase), or the araBAD operon. Often, such prokaryotic expression vectors will further include transcription 25 terminators, such as the aspA terminator, and elements that facilitate translation, such as a consensus ribosome binding site and translation termination codon, Schomer et al., *Proc. Natl. Acad. Sci. USA* 83:8506-8510 (1986).

As another example, vectors for expressing 30 proteins of the present invention in yeast cells, typically *S. cerevisiae*, will include a yeast promoter, such as the CYC1 promoter, the GAL1 promoter, ADH1 promoter, or the GPD promoter, and will typically have elements that facilitate

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transcription termination, such as the transcription termination signals from the CYC1 or ADH1 gene.

As another example, vectors for expressing proteins of the present invention in mammalian cells will include a promoter active in mammalian cells. Such promoters are often drawn from mammalian viruses - such as the enhancer-promoter sequences from the immediate early gene of the human cytomegalovirus (CMV), the enhancer-promoter sequences from the Rous sarcoma virus long terminal repeat (RSV LTR), and the enhancer-promoter from SV40. Often, expression is enhanced by incorporation of polyadenylation sites, such as the late SV40 polyadenylation site and the polyadenylation signal and transcription termination sequences from the bovine growth hormone (BGH) gene, and ribosome binding sites. Furthermore, vectors can include introns, such as intron II of rabbit β -globin gene and the SV40 splice elements.

Vector-drive protein expression can be constitutive or inducible.

Inducible vectors include either naturally inducible promoters, such as the trc promoter, which is regulated by the lac operon, and the pL promoter, which is regulated by tryptophan, the MMTV-LTR promoter, which is inducible by dexamethasone, or can contain synthetic promoters and/or additional elements that confer inducible control on adjacent promoters. Examples of inducible synthetic promoters are the hybrid Plac/ara-1 promoter and the PLtetO-1 promoter. The PLtetO-1 promoter takes advantage of the high expression levels from the PL promoter of phage lambda, but replaces the lambda repressor sites with two copies of operator 2 of the Tn10 tetracycline resistance operon, causing this promoter to be tightly repressed by the Tet repressor protein and induced in

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response to tetracycline (Tc) and Tc derivatives such as anhydrotetracycline.

As another example of inducible elements, hormone response elements, such as the glucocorticoid response element (GRE) and the estrogen response element (ERE), can
5 confer hormone inducibility where vectors are used for expression in cells having the respective hormone receptors.

To reduce background levels of expression, elements responsive to ecdysone, an insect hormone, can be used
10 instead, with coexpression of the ecdysone receptor.

Expression vectors can be designed to fuse the expressed polypeptide to small protein tags that facilitate purification and/or visualization.

For example, proteins of the present invention can
15 be expressed with a polyhistidine tag that facilitates purification of the fusion protein by immobilized metal affinity chromatography, for example using NiNTA resin (Qiagen Inc., Valencia, CA, USA) or TALONTM resin (cobalt immobilized affinity chromatography medium, Clontech Labs,
20 Palo Alto, CA, USA). As another example, the fusion protein can include a chitin-binding tag and self-excising intein, permitting chitin-based purification with self-removal of the fused tag (IMPACTTM system, New England Biolabs, Inc., Beverley, MA, USA). Alternatively, the fusion protein can
25 include a calmodulin-binding peptide tag, permitting purification by calmodulin affinity resin (Stratagene, La Jolla, CA, USA), or a specifically excisable fragment of the biotin carboxylase carrier protein, permitting purification of *in vivo* biotinylated protein using an avidin resin and
30 subsequent tag removal (Promega, Madison, WI, USA). As another useful alternative, the proteins of the present invention can be expressed as a fusion to glutathione-S-

transferase, the affinity and specificity of binding to glutathione permitting purification using glutathione affinity resins, such as Glutathione-Superflow Resin (Clontech Laboratories, Palo Alto, CA, USA), with subsequent
5 elution with free glutathione.

Other tags include, for example, the Xpress epitope, detectable by anti-Xpress antibody (Invitrogen, Carlsbad, CA, USA), a myc tag, detectable by anti-myc tag antibody, the V5 epitope, detectable by anti-V5 antibody
10 (Invitrogen, Carlsbad, CA, USA), FLAG® epitope, detectable by anti-FLAG® antibody (Stratagene, La Jolla, CA, USA), and the HA epitope.

For secretion of expressed proteins, vectors can include appropriate sequences that encode secretion signals, such as leader peptides. For example, the pSecTag2 vectors
15 (Invitrogen, Carlsbad, CA, USA) are 5.2 kb mammalian expression vectors that carry the secretion signal from the V-J2-C region of the mouse Ig kappa-chain for efficient secretion of recombinant proteins from a variety of
20 mammalian cell lines.

Expression vectors can also be designed to fuse proteins encoded by the heterologous nucleic acid insert to polypeptides larger than purification and/or identification tags. Useful protein fusions include those that permit
25 display of the encoded protein on the surface of a phage or cell, fusions to intrinsically fluorescent proteins, such as those that have a green fluorescent protein (GFP)-like chromophore, fusions to the IgG Fc region, and fusions for use in two hybrid systems.

30 Vectors for phage display fuse the encoded polypeptide to, e.g., the gene III protein (pIII) or gene VIII protein (pVIII) for display on the surface of filamentous phage, such as M13. See Barbas *et al.*, Phage

Display: A Laboratory Manual, Cold Spring Harbor Laboratory Press (2001) (ISBN 0-87969-546-3); Kay *et al.* (eds.), Phage Display of Peptides and Proteins: A Laboratory Manual, San Diego: Academic Press, Inc., 1996; Abelson *et al.* (eds.),
5 Combinatorial Chemistry, Methods in Enzymology vol. 267, Academic Press (May 1996).

Vectors for yeast display, e.g. the pYD1 yeast display vector (Invitrogen, Carlsbad, CA, USA), use the α -agglutinin yeast adhesion receptor to display recombinant
10 protein on the surface of *S. cerevisiae*. Vectors for mammalian display, e.g., the pDisplay™ vector (Invitrogen, Carlsbad, CA, USA), target recombinant proteins using an N-terminal cell surface targeting signal and a C-terminal transmembrane anchoring domain of platelet derived growth
15 factor receptor.

A wide variety of vectors now exist that fuse proteins encoded by heterologous nucleic acids to the chromophore of the substrate-independent, intrinsically fluorescent green fluorescent protein from *Aequorea victoria*
20 ("GFP") and its variants. These proteins are intrinsically fluorescent: the GFP-like chromophore is entirely encoded by its amino acid sequence and can fluoresce without requirement for cofactor or substrate.

Structurally, the GFP-like chromophore comprises
25 an 11-stranded β -barrel (β -can) with a central α -helix, the central α -helix having a conjugated π -resonance system that includes two aromatic ring systems and the bridge between them. The π -resonance system is created by autocatalytic cyclization among amino acids; cyclization proceeds through
30 an imidazolinone intermediate, with subsequent dehydrogenation by molecular oxygen at the C α -C β bond of a participating tyrosine.

The GFP-like chromophore can be selected from GFP-like chromophores found in naturally occurring proteins, such as *A. victoria* GFP (GenBank accession number AAA27721), *Renilla reniformis* GFP, FP583 (GenBank accession no.

5 AF168419) (DsRed), FP593 (AF272711), FP483 (AF168420), FP484 (AF168424), FP595 (AF246709), FP486 (AF168421), FP538 (AF168423), and FP506 (AF168422), and need include only so much of the native protein as is needed to retain the chromophore's intrinsic fluorescence. Methods for
10 determining the minimal domain required for fluorescence are known in the art. Li et al., "Deletions of the *Aequorea victoria* Green Fluorescent Protein Define the Minimal Domain Required for Fluorescence," *J. Biol. Chem.* 272:28545-28549 (1997).

15 Alternatively, the GFP-like chromophore can be selected from GFP-like chromophores modified from those found in nature. Typically, such modifications are made to improve recombinant production in heterologous expression systems (with or without change in protein sequence), to
20 alter the excitation and/or emission spectra of the native protein, to facilitate purification, to facilitate or as a consequence of cloning, or are a fortuitous consequence of research investigation.

The methods for engineering such modified GFP-like
25 chromophores and testing them for fluorescence activity, both alone and as part of protein fusions, are well-known in the art. Early results of these efforts are reviewed in Heim et al., *Curr. Biol.* 6:178-182 (1996), incorporated herein by reference in its entirety; a more recent review,
30 with tabulation of useful mutations, is found in Palm et al., "Spectral Variants of Green Fluorescent Protein," in Green Fluorescent Proteins, Conn (ed.), *Methods Enzymol.* vol. 302, pp. 378 - 394 (1999), incorporated herein by

reference in its entirety. A variety of such modified chromophores are now commercially available and can readily be used in the fusion proteins of the present invention.

For example, EGFP ("enhanced GFP"), Cormack et al., *Gene* 173:33-38 (1996); U.S. Pat. Nos. 6,090,919 and 5,804,387, is a red-shifted, human codon-optimized variant of GFP that has been engineered for brighter fluorescence, higher expression in mammalian cells, and for an excitation spectrum optimized for use in flow cytometers. EGFP can usefully contribute a GFP-like chromophore to the fusion proteins of the present invention. A variety of EGFP vectors, both plasmid and viral, are available commercially (Clontech Labs, Palo Alto, CA, USA), including vectors for bacterial expression, vectors for N-terminal protein fusion expression, vectors for expression of C-terminal protein fusions, and for bicistronic expression.

Toward the other end of the emission spectrum, EBFP ("enhanced blue fluorescent protein") and BFP2 contain four amino acid substitutions that shift the emission from green to blue, enhance the brightness of fluorescence and improve solubility of the protein, Heim et al., *Curr. Biol.* 6:178-182 (1996); Cormack et al., *Gene* 173:33-38 (1996). EBFP is optimized for expression in mammalian cells whereas BFP2, which retains the original jellyfish codons, can be expressed in bacteria; as is further discussed below, the host cell of production does not affect the utility of the resulting fusion protein. The GFP-like chromophores from EBFP and BFP2 can usefully be included in the fusion proteins of the present invention, and vectors containing these blue-shifted variants are available from Clontech Labs (Palo Alto, CA, USA).

Analogously, EYFP ("enhanced yellow fluorescent protein"), also available from Clontech Labs, contains four

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amino acid substitutions, different from EBFP, Ormö *et al.*,
Science 273:1392-1395 (1996), that shift the emission from
green to yellowish-green. Citrine, an improved yellow
fluorescent protein mutant, is described in Heikal *et al.*,
5 *Proc. Natl. Acad. Sci. USA* 97:11996-12001 (2000). ECFP
("enhanced cyan fluorescent protein") (Clontech Labs, Palo
Alto, CA, USA) contains six amino acid substitutions, one of
which shifts the emission spectrum from green to cyan. Heim
et al., *Curr. Biol.* 6:178-182 (1996); Miyawaki *et al.*,
10 *Nature* 388:882-887 (1997). The GFP-like chromophore of each
of these GFP variants can usefully be included in the fusion
proteins of the present invention.

The GFP-like chromophore can also be drawn from
other modified GFPs, including those described in U.S. Pat.
15 Nos. 6,124,128; 6,096,865; 6,090,919; 6,066,476; 6,054,321;
6,027,881; 5,968,750; 5,874,304; 5,804,387; 5,777,079;
5,741,668; and 5,625,048, the disclosures of which are
incorporated herein by reference in their entireties. See
also Conn (ed.), Green Fluorescent Protein, *Methods in*
20 *Enzymol.* Vol. 302, pp 378-394 (1999), incorporated herein by
reference in its entirety. A variety of such modified
chromophores are now commercially available and can readily
be used in the fusion proteins of the present invention.

Fusions to the IgG Fc region increase serum half
25 life of protein pharmaceutical products through interaction
with the FcRn receptor (also denominated the FcRp receptor
and the Brambell receptor, FcRb), further described in
international patent application nos. WO 97/43316, WO
97/34631, WO 96/32478, WO 96/18412.

30 For long-term, high-yield recombinant production
of the proteins, protein fusions, and protein fragments of
the present invention, stable expression is particularly
useful.

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Stable expression is readily achieved by integration into the host cell genome of vectors having selectable markers, followed by selection for integrants.

For example, the pUB6/V5-His A, B, and C vectors
5 (Invitrogen, Carlsbad, CA, USA) are designed for high-level stable expression of heterologous proteins in a wide range of mammalian tissue types and cell lines. pUB6/V5-His uses the promoter/enhancer sequence from the human ubiquitin C gene to drive expression of recombinant proteins: expression
10 levels in 293, CHO, and NIH3T3 cells are comparable to levels from the CMV and human EF-1a promoters. The bsd gene permits rapid selection of stably transfected mammalian cells with the potent antibiotic blasticidin.

Replication incompetent retroviral vectors,
15 typically derived from Moloney murine leukemia virus, prove particularly useful for creating stable transfectants having integrated provirus. The highly efficient transduction machinery of retroviruses, coupled with the availability of a variety of packaging cell lines - such as RetroPack™ PT
20 67, EcoPack2™-293, AmphoPack-293, GP2-293 cell lines (all available from Clontech Laboratories, Palo Alto, CA, USA) - allow a wide host range to be infected with high efficiency; varying the multiplicity of infection readily adjusts the copy number of the integrated provirus. Retroviral vectors
25 are available with a variety of selectable markers, such as resistance to neomycin, hygromycin, and puromycin, permitting ready selection of stable integrants.

The present invention further includes host cells
30 comprising the vectors of the present invention, either present episomally within the cell or integrated, in whole or in part, into the host cell chromosome.

Among other considerations, some of which are described above, a host cell strain may be chosen for its ability to process the expressed protein in the desired fashion. Such post-translational modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation, and it is an aspect of the present invention to provide MDZ3, MDZ4, MDZ7 and MDZ12 proteins, respectively, with such post-translational modifications.

As noted earlier, host cells can be prokaryotic or eukaryotic. Representative examples of appropriate host cells include, but are not limited to, bacterial cells, such as *E. coli*, *Caulobacter crescentus*, *Streptomyces* species, and *Salmonella typhimurium*; yeast cells, such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Pichia pastoris*, *Pichia methanolica*; insect cell lines, such as those from *Spodoptera frugiperda* - e.g., Sf9 and Sf21 cell lines, and expressTM cells (Protein Sciences Corp., Meriden, CT, USA) - *Drosophila* S2 cells, and *Trichoplusia ni* High Five® Cells (Invitrogen, Carlsbad, CA, USA); and mammalian cells. Typical mammalian cells include COS1 and COS7 cells, chinese hamster ovary (CHO) cells, NIH 3T3 cells, 293 cells, HEPG2 cells, HeLa cells, L cells, murine ES cell lines (e.g., from strains 129/SV, C57/BL6, DBA-1, 129/SVJ), K562, Jurkat cells, and BW5147. Other mammalian cell lines are well known and readily available from the American Type Culture Collection (ATCC) (Manassas, VA, USA) and the National Institute of General medical Sciences (NIGMS) Human Genetic Cell Repository at the Coriell Cell Repositories (Camden, NJ, USA).

Methods for introducing the vectors and nucleic acids of the present invention into the host cells are well known in the art; the choice of technique will depend

primarily upon the specific vector to be introduced and the host cell chosen.

For example, phage lambda vectors will typically be packaged using a packaging extract (e.g., Gigapack® packaging extract, Stratagene, La Jolla, CA, USA), and the packaged virus used to infect *E. coli*. Plasmid vectors will typically be introduced into chemically competent or electrocompetent bacterial cells.

E. coli cells can be rendered chemically competent by treatment, e.g., with CaCl_2 , or a solution of Mg^{2+} , Mn^{2+} , Ca^{2+} , Rb^+ or K^+ , dimethyl sulfoxide, dithiothreitol, and hexamine cobalt (III), Hanahan, *J. Mol. Biol.* 166(4):557-80 (1983), and vectors introduced by heat shock. A wide variety of chemically competent strains are also available commercially (e.g., Epicurian Coli® XL10-Gold® Ultracompetent Cells (Stratagene, La Jolla, CA, USA); DH5α competent cells (Clontech Laboratories, Palo Alto, CA, USA); TOP10 Chemically Competent *E. coli* Kit (Invitrogen, Carlsbad, CA, USA)).

Bacterial cells can be rendered electrocompetent – that is, competent to take up exogenous DNA by electroporation – by various pre-pulse treatments; vectors are introduced by electroporation followed by subsequent outgrowth in selected media. An extensive series of protocols is provided online in Electroprotocols (BioRad, Richmond, CA, USA) (http://www.biorad.com/LifeScience/pdf/New_Gene_Pulser.pdf).

Vectors can be introduced into yeast cells by spheroplasting, treatment with lithium salts, electroporation, or protoplast fusion.

Spheroplasts are prepared by the action of hydrolytic enzymes – a snail-gut extract, usually denoted Glusulase, or Zymolyase, an enzyme from *Arthrobacter*

luteus - to remove portions of the cell wall in the presence of osmotic stabilizers, typically 1 M sorbitol. DNA is added to the spheroplasts, and the mixture is co-precipitated with a solution of polyethylene glycol (PEG) and Ca^{2+} . Subsequently, the cells are resuspended in a solution of sorbitol, mixed with molten agar and then layered on the surface of a selective plate containing sorbitol. For lithium-mediated transformation, yeast cells are treated with lithium acetate, which apparently permeabilizes the cell wall, DNA is added and the cells are co-precipitated with PEG. The cells are exposed to a brief heat shock, washed free of PEG and lithium acetate, and subsequently spread on plates containing ordinary selective medium. Increased frequencies of transformation are obtained by using specially-prepared single-stranded carrier DNA and certain organic solvents. Schiestl *et al.*, *Curr. Genet.* 16(5-6):339-46 (1989). For electroporation, freshly-grown yeast cultures are typically washed, suspended in an osmotic protectant, such as sorbitol, mixed with DNA, and the cell suspension pulsed in an electroporation device. Subsequently, the cells are spread on the surface of plates containing selective media. Becker *et al.*, *Methods Enzymol.* 194:182-7 (1991). The efficiency of transformation by electroporation can be increased over 100-fold by using PEG, single-stranded carrier DNA and cells that are in late log-phase of growth. Larger constructs, such as YACs, can be introduced by protoplast fusion.

Mammalian and insect cells can be directly infected by packaged viral vectors, or transfected by chemical or electrical means.

For chemical transfection, DNA can be coprecipitated with CaPO_4 or introduced using liposomal and nonliposomal lipid-based agents. Commercial kits are

available for CaPO₄ transfection (CalPhosTM Mammalian Transfection Kit, Clontech Laboratories, Palo Alto, CA, USA), and lipid-mediated transfection can be practiced using commercial reagents, such as LIPOFECTAMINETM 2000,

- 5 LIPOFECTAMINETM Reagent, CELLFECTIN[®] Reagent, and LIPOFECTIN[®] Reagent (Invitrogen, Carlsbad, CA, USA), DOTAP Liposomal Transfection Reagent, FuGENE 6, X-tremeGENE Q2, DOSPER, (Roche Molecular Biochemicals, Indianapolis, IN USA), EffecteneTM, PolyFect[®], Superfect[®] (Qiagen, Inc.,
- 10 Valencia, CA, USA). Protocols for electroporating mammalian cells can be found online in Electroprotocols (Bio-Rad, Richmond, CA, USA) (http://www.bio-rad.com/LifeScience/pdf/New_Gene_Pulser.pdf). See also, Norton et al. (eds.), Gene Transfer Methods: Introducing DNA into Living Cells and Organisms,
- 15 BioTechniques Books, Eaton Publishing Co. (2000) (ISBN 1-881299-34-1), incorporated herein by reference in its entirety.

- Other transfection techniques include transfection
- 20 by particle bombardment. See, e.g., Cheng et al., *Proc. Natl. Acad. Sci. USA* 90(10):4455-9 (1993); Yang et al., *Proc. Natl. Acad. Sci. USA* 87(24):9568-72 (1990).

PROTEINS

25

- In another aspect, the present invention provides MDZ3, MDZ4, MDZ7 and MDZ12 proteins, respectively, various fragments thereof suitable for use as antigens (e.g., for epitope mapping) and for use as immunogens (e.g., for
- 30 raising antibodies or as vaccines), fusions of MDZ3, MDZ4, MDZ7 and MDZ12 polypeptides and fragments to heterologous polypeptides, and conjugates of the proteins, fragments, and

fusions of the present invention to other moieties (e.g., to carrier proteins, to fluorophores).

FIGS. 3, 6, 9, 12 and 13 present the predicted amino acid sequences encoded by the MDZ3, MDZ4, MDZ7, MDZ12a and MDZ12b (S and L) cDNA clones, respectively. The amino acid sequences are further presented, respectively, in SEQ ID Nos: 3, 3029, 4409, 5772, 6939 and 6940.

Unless otherwise indicated, amino acid sequences of the proteins of the present invention were determined as a predicted translation from a nucleic acid sequence. Accordingly, any amino acid sequence presented herein may contain errors due to errors in the nucleic acid sequence, as described in detail above. Furthermore, single nucleotide polymorphisms (SNPs) occur frequently in eukaryotic genomes - more than 1.4 million SNPs have already identified in the human genome, International Human Genome Sequencing Consortium, Nature 409:860 - 921 (2001) - and the sequence determined from one individual of a species may differ from other allelic forms present within the population. Small deletions and insertions can often be found that do not alter the function of the protein.

Accordingly, it is an aspect of the present invention to provide proteins not only identical in sequence to those described with particularity herein, but also to provide isolated proteins at least about 65% identical in sequence to those described with particularity herein, typically at least about 70%, 75%, 80%, 85%, or 90% identical in sequence to those described with particularity herein, usefully at least about 91%, 92%, 93%, 94%, or 95% identical in sequence to those described with particularity herein, usefully at least about 96%, 97%, 98%, or 99% identical in sequence to those described with particularity herein, and, most conservatively, at least about 99.5%,

99.6%, 99.7%, 99.8% and 99.9% identical in sequence to those described with particularity herein. These sequence variants can be naturally occurring or can result from human intervention by way of random or directed mutagenesis.

5 For purposes herein, percent identity of two amino acid sequences is determined using the procedure of Tatiana *et al.*, "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", *FEMS Microbiol Lett.* 174:247-250 (1999), which procedure is effectuated by the
10 computer program BLAST 2 SEQUENCES, available online at

<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>,

To assess percent identity of amino acid sequences, the
15 BLASTP module of BLAST 2 SEQUENCES is used with default values of (i) BLOSUM62 matrix, Henikoff *et al.*, *Proc. Natl. Acad. Sci USA* 89(22):10915-9 (1992); (ii) open gap 11 and extension gap 1 penalties; and (iii) gap x_dropoff 50 expect 10 word size 3 filter, and both sequences are entered in
20 their entireties.

As is well known, amino acid substitutions occur frequently among natural allelic variants, with conservative substitutions often occasioning only *de minimis* change in protein function.

25 Accordingly, it is an aspect of the present invention to provide proteins not only identical in sequence to those described with particularity herein, but also to provide isolated proteins having the sequence of MDZ3, MDZ4, MDZ7 and MDZ12 proteins, respectively, or portions thereof,
30 with conservative amino acid substitutions. It is a further aspect to provide isolated proteins having the sequence of MDZ3, MDZ4, MDZ7 and MDZ12 proteins, respectively, and portions thereof, with moderately conservative amino acid

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substitutions. These conservatively-substituted and moderately conservatively-substituted variants can be naturally occurring or can result from human intervention.

Although there are a variety of metrics for
 5 calling conservative amino acid substitutions, based primarily on either observed changes among evolutionarily related proteins or on predicted chemical similarity, for purposes herein a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix
 10 reproduced herein below (see Gonnet *et al.*, *Science* 256(5062):1443-5 (1992)):

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
	A	2	-1	0	0	0	0	0	-1	-1	-1	0	-1	-2	0	1	1	-4	-2	0
15	R	-1	5	0	0	-2	2	0	-1	1	-2	3	-2	-3	-1	0	0	-2	-2	-2
	N	0	0	4	2	-2	1	1	0	1	-3	-3	1	-2	-3	-1	1	0	-4	-1
	D	0	0	2	5	-3	1	3	0	0	-4	-4	0	-3	-4	-1	0	0	-5	-3
	C	0	-2	-2	-3	12	-2	-3	-2	-1	-1	-2	-3	-1	-1	-3	0	0	-1	0
	Q	0	2	1	1	-2	3	2	-1	1	-2	-2	2	-1	-3	0	0	0	-3	-2
20	E	0	0	1	3	-3	2	4	-1	0	-3	-3	1	-2	-4	0	0	0	-4	-3
	G	0	-1	0	0	-2	-1	-1	7	-1	-4	-4	-1	-4	-5	-2	0	-1	-4	-4
	H	-1	1	1	0	-1	1	0	-1	6	-2	-2	1	-1	0	-1	0	0	-1	2
	I	-1	-2	-3	-4	-1	-2	-3	-4	-2	4	3	-2	2	1	-3	-2	-1	-2	-1
	L	-1	-2	-3	-4	-2	-2	-3	-4	-2	3	4	-2	3	2	-2	-2	-1	-1	0
25	K	0	3	1	0	-3	2	1	-1	1	-2	-2	3	-1	-3	-1	0	0	-4	-2
	M	-1	-2	-2	-3	-1	-1	-2	-4	-1	2	3	-1	4	2	-2	-1	-1	-1	0
	F	-2	-3	-3	-4	-1	-3	-4	-5	0	1	2	-3	2	7	-4	-3	-2	4	5
	P	0	-1	-1	-1	-3	0	0	-2	-1	-3	-2	-1	-2	-4	8	0	0	-5	-3
	S	1	0	1	0	0	0	0	0	-2	-2	0	-1	-3	0	2	2	-3	-2	-1
30	T	1	0	0	0	0	0	0	-1	0	-1	-1	0	-1	-2	0	2	2	-4	-2
	W	-4	-2	-4	-5	-1	-3	-4	-4	-1	-2	-1	-4	-1	4	-5	-3	-4	14	4
	Y	-2	-2	-1	-3	0	-2	-3	-4	2	-1	0	-2	0	5	-3	-2	-2	4	8
	V	0	-2	-2	-3	0	-2	-2	-3	-2	3	2	-2	2	0	-2	-1	0	-3	-1

For purposes herein, a "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix reproduced herein above.

As is also well known in the art, relatedness of
5 proteins can also be characterized using a functional test, the ability of the encoding nucleic acids to base-pair to one another at defined hybridization stringencies.

It is, therefore, another aspect of the invention to provide isolated proteins not only identical in sequence
10 to those described with particularity herein, but also to provide isolated proteins ("hybridization related proteins") that are encoded by nucleic acids that hybridize under high stringency conditions (as defined herein above) to all or to a portion of various of the isolated nucleic acids of the
15 present invention ("reference nucleic acids"). It is a further aspect of the invention to provide isolated proteins ("hybridization related proteins") that are encoded by nucleic acids that hybridize under moderate stringency conditions (as defined herein above) to all or to a portion
20 of various of the isolated nucleic acids of the present invention ("reference nucleic acids").

The hybridization related proteins can be alternative isoforms, homologues, paralogues, and orthologues of the MDZ3, MDZ4, MDZ7 and MDZ12 proteins,
25 respectively, of the present invention. Particularly useful orthologues are those from other primate species, such as chimpanzee, rhesus macaque monkey, baboon, orangutan, and gorilla, from rodents, such as rats, mice, guinea pigs; from lagomorphs, such as rabbits, and from domestic livestock,
30 such as cow, pig, sheep, horse, and goat.

Relatedness of proteins can also be characterized using a second functional test, the ability of a first

protein competitively to inhibit the binding of a second protein to an antibody.

It is, therefore, another aspect of the present invention to provide isolated proteins not only identical in sequence to those described with particularity herein, but also to provide isolated proteins ("cross-reactive proteins") that competitively inhibit the binding of antibodies to all or to a portion of various of the isolated MDZ3, MDZ4, MDZ7 and MDZ12 proteins, respectively, of the present invention ("reference proteins"). Such competitive inhibition can readily be determined using immunoassays well known in the art.

Among the proteins of the present invention that differ in amino acid sequence from those described with particularity herein -- including those that have deletions and insertions causing up to 10% non-identity, those having conservative or moderately conservative substitutions, hybridization related proteins, and cross-reactive proteins -- those that substantially retain one or more MDZ3, MDZ4, MDZ7 or MDZ12 activities are particularly useful. As described above, those activities include transcription regulation and protein-protein interaction.

Residues that are tolerant of change while retaining function can be identified by altering the protein at known residues using methods known in the art, such as alanine scanning mutagenesis, Cunningham *et al.*, *Science* 244(4908):1081-5 (1989); transposon linker scanning mutagenesis, Chen *et al.*, *Gene* 263(1-2):39-48 (2001); combinations of homolog- and alanine-scanning mutagenesis, Jin *et al.*, *J. Mol. Biol.* 226(3):851-65 (1992); combinatorial alanine scanning, Weiss *et al.*, *Proc. Natl. Acad. Sci USA* 97(16):8950-4 (2000), followed by functional assay. Transposon linker scanning kits are available

commercially (New England Biolabs, Beverly, MA, USA,
catalog. no. E7-102S; EZ::TN™ In-Frame Linker Insertion
Kit, catalogue no. EZI04KN, Epicentre Technologies
Corporation, Madison, WI, USA).

5 As further described below, the isolated proteins
of the present invention can readily be used as specific
immunogens to raise antibodies that specifically recognize
MDZ3, MDZ4, MDZ7 or MDZ12 (MDZ12a, MDZ12bS, MDZ12bL)
proteins, their isoforms, homologues, paralogues, and/or
10 orthologues. The antibodies, in turn, can be used, *inter*
alia, specifically to assay for the MDZ3, MDZ4, MDZ7 or
MDZ12 proteins of the present invention — e.g. by ELISA for
detection of protein fluid samples, such as serum, by
immunohistochemistry or laser scanning cytometry, for
15 detection of protein in tissue samples, or by flow
cytometry, for detection of intracellular protein in cell
suspensions — for specific antibody-mediated isolation
and/or purification of MDZ3, MDZ4, MDZ7 or MDZ12 proteins,
as for example by immunoprecipitation, and for use as
20 specific agonists or antagonists of MDZ3, MDZ4, MDZ7 or
MDZ12 action.

The isolated proteins of the present invention are
also immediately available for use as specific standards in
assays used to determine the concentration and/or amount
25 specifically of the MDZ3, MDZ4, MDZ7 or MDZ12 proteins of
the present invention. As is well known, ELISA kits for
detection and quantitation of protein analytes typically
include isolated and purified protein of known concentration
for use as a measurement standard (e.g., the human
30 interferon- γ OptEIA kit, catalog no. 555142, Pharmingen, San
Diego, CA, USA includes human recombinant gamma interferon,
baculovirus produced).

The isolated proteins of the present invention are also immediately available for use as specific biomolecule capture probes for surface-enhanced laser desorption ionization (SELDI) detection of protein-protein
5 interactions, WO 98/59362; WO 98/59360; WO 98/59361; and Merchant *et al.*, *Electrophoresis* 21(6):1164-77 (2000), the disclosures of which are incorporated herein by reference in their entirety. Analogously, the isolated proteins of the present invention are also immediately available for use as
10 specific biomolecule capture probes on BIACORE surface plasmon resonance probes. See Weinberger *et al.*, *Pharmacogenomics* 1(4):395-416 (2000); Malmqvist, *Biochem. Soc. Trans.* 27(2):335-40 (1999).

The isolated proteins of the present invention are
15 also useful as a therapeutic supplement in patients having a specific deficiency in MDZ3, MDZ4, MDZ7 or MDZ12 production, respectively.

In another aspect, the invention also provides fragments of various of the proteins of the present
20 invention. The protein fragments are useful, *inter alia*, as antigenic and immunogenic fragments of MDZ3, MDZ4, MDZ7 or MDZ12, respectively.

By "fragments" of a protein is here intended isolated proteins (equally, polypeptides, peptides,
25 oligopeptides), however obtained, that have an amino acid sequence identical to a portion of the reference amino acid sequence, which portion is at least 6 amino acids and less than the entirety of the reference nucleic acid. As so defined, "fragments" need not be obtained by physical
30 fragmentation of the reference protein, although such provenance is not thereby precluded.

Fragments of at least 6 contiguous amino acids are useful in mapping B cell and T cell epitopes of the

reference protein. See, e.g., Geysen et al., "Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid," *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1984) and U.S. Pat. Nos. 4,708,871 and 5,595,915, the disclosures of which are incorporated herein by reference in their entireties. Because the fragment need not itself be immunogenic, part of an immunodominant epitope, nor even recognized by native antibody, to be useful in such epitope mapping, all fragments of at least 6 amino acids of the proteins of the present invention have utility in such a study.

Fragments of at least 8 contiguous amino acids, often at least 15 contiguous amino acids, have utility as immunogens for raising antibodies that recognize the proteins of the present invention. See, e.g., Lerner, "Tapping the immunological repertoire to produce antibodies of predetermined specificity," *Nature* 299:592-596 (1982); Shinnick et al., "Synthetic peptide immunogens as vaccines," *Annu. Rev. Microbiol.* 37:425-46 (1983); Sutcliffe et al., "Antibodies that react with predetermined sites on proteins," *Science* 219:660-6 (1983), the disclosures of which are incorporated herein by reference in their entireties. As further described in the above-cited references, virtually all 8-mers, conjugated to a carrier, such as a protein, prove immunogenic - that is, prove capable of eliciting antibody for the conjugated peptide; accordingly, all fragments of at least 8 amino acids of the proteins of the present invention have utility as immunogens.

Fragments of at least 8, 9, 10 or 12 contiguous amino acids are also useful as competitive inhibitors of binding of the entire protein, or a portion thereof, to antibodies (as in epitope mapping), and to natural binding

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partners, such as subunits in a multimeric complex or to
receptors or ligands of the subject protein; this
competitive inhibition permits identification and separation
of molecules that bind specifically to the protein of
5 interest, U.S. Pat. Nos. 5,539,084 and 5,783,674,
incorporated herein by reference in their entireties.

The protein, or protein fragment, of the present
invention is thus at least 6 amino acids in length,
typically at least 8, 9, 10 or 12 amino acids in length, and
10 often at least 15 amino acids in length. Often, the protein
or the present invention, or fragment thereof, is at least
20 amino acids in length, even 25 amino acids, 30 amino
acids, 35 amino acids, or 50 amino acids or more in length.

Of course, larger fragments having at least 75 amino acids,
15 100 amino acids, or even 150 amino acids are also useful,
and at times preferred.

The present invention further provides fusions of
each of the proteins and protein fragments of the present
invention to heterologous polypeptides.

20 By fusion is here intended that the protein or
protein fragment of the present invention is linearly
contiguous to the heterologous polypeptide in a peptide-
bonded polymer of amino acids or amino acid analogues; by
"heterologous polypeptide" is here intended a polypeptide
25 that does not naturally occur in contiguity with the protein
or protein fragment of the present invention. As so
defined, the fusion can consist entirely of a plurality of
fragments of any one of the MDZ3, MDZ4, MDZ7 or MDZ12
proteins, respectively, in altered arrangement; in such
30 case, any of the MDZ3, MDZ4, MDZ7 or MDZ12 fragments can be
considered heterologous to the other MDZ3, MDZ4, MDZ7 or
MDZ12 fragments in the fusion protein. More typically,

however, the heterologous polypeptide is not drawn from the MDZ3, MDZ4, MDZ7 or MDZ12 protein itself.

The fusion proteins of the present invention will include at least one fragment of the protein of the present invention, which fragment is at least 6, typically at least 8, often at least 15, and usefully at least 16, 17, 18, 19, or 20 amino acids long. The fragment of the protein of the present to be included in the fusion can usefully be at least 25 amino acids long, at least 50 amino acids long, and can be at least 75, 100, or even 150 amino acids long. Fusions that include the entirety of the proteins of the present invention have particular utility.

The heterologous polypeptide included within the fusion protein of the present invention is at least 6 amino acids in length, often at least 8 amino acids in length, and usefully at least 15, 20, and 25 amino acids in length. Fusions that include larger polypeptides, such as the IgG Fc region, and even entire proteins (such as GFP chromophore-containing proteins), have particular utility.

As described above in the description of vectors and expression vectors of the present invention, which discussion is incorporated herein by reference in its entirety, heterologous polypeptides to be included in the fusion proteins of the present invention can usefully include those designed to facilitate purification and/or visualization of recombinantly-expressed proteins. Although purification tags can also be incorporated into fusions that are chemically synthesized, chemical synthesis typically provides sufficient purity that further purification by HPLC suffices; however, visualization tags as above described retain their utility even when the protein is produced by chemical synthesis, and when so included render the fusion

proteins of the present invention useful as directly detectable markers of MDZ3, MDZ4, MDZ7 or MDZ12 presence.

As also discussed above, heterologous polypeptides to be included in the fusion proteins of the present invention can usefully include those that facilitate secretion of recombinantly expressed proteins - into the periplasmic space or extracellular milieu for prokaryotic hosts, into the culture medium for eukaryotic cells - through incorporation of secretion signals and/or leader sequences.

Other useful protein fusions of the present invention include those that permit use of the protein of the present invention as bait in a yeast two-hybrid system.

See Bartel et al. (eds.), The Yeast Two-Hybrid System, Oxford University Press (1997) (ISBN: 0195109384); Zhu et al., Yeast Hybrid Technologies, Eaton Publishing, (2000) (ISBN 1-881299-15-5); Fields et al., *Trends Genet.* 10(8):286-92 (1994); Mendelsohn et al., *Curr. Opin. Biotechnol.* 5(5):482-6 (1994); Luban et al., *Curr. Opin. Biotechnol.* 6(1):59-64 (1995); Allen et al., *Trends Biochem. Sci.* 20(12):511-6 (1995); Drees, *Curr. Opin. Chem. Biol.* 3(1):64-70 (1999); Topcu et al., *Pharm. Res.* 17(9):1049-55 (2000); Fashena et al., *Gene* 250(1-2):1-14 (2000), the disclosures of which are incorporated herein by reference in their entirety. Typically, such fusion is to either *E. coli* LexA or yeast GAL4 DNA binding domains. Related bait plasmids are available that express the bait fused to a nuclear localization signal.

Other useful protein fusions include those that permit display of the encoded protein on the surface of a phage or cell, fusions to intrinsically fluorescent proteins, such as green fluorescent protein (GFP), and fusions to the IgG Fc region, as described above, which

discussion is incorporated here by reference in its entirety.

5 The proteins and protein fragments of the present invention can also usefully be fused to protein toxins, such as *Pseudomonas* exotoxin A, diphtheria toxin, shiga toxin A, anthrax toxin lethal factor, ricin, in order to effect ablation of cells that bind or take up the proteins of the present invention.

10 The isolated proteins, protein fragments, and protein fusions of the present invention can be composed of natural amino acids linked by native peptide bonds, or can contain any or all of nonnatural amino acid analogues, nonnative bonds, and post-synthetic (post translational) modifications, either throughout the length of the protein
15 or localized to one or more portions thereof.

20 As is well known in the art, when the isolated protein is used, e.g., for epitope mapping, the range of such nonnatural analogues, nonnative inter-residue bonds, or post-synthesis modifications will be limited to those that permit binding of the peptide to antibodies. When used as an immunogen for the preparation of antibodies in a non-human host, such as a mouse, the range of such nonnatural analogues, nonnative inter-residue bonds, or post-synthesis
25 modifications will be limited to those that do not interfere with the immunogenicity of the protein. When the isolated protein is used as a therapeutic agent, such as a vaccine or for replacement therapy, the range of such changes will be limited to those that do not confer toxicity upon the isolated protein.

30 Non-natural amino acids can be incorporated during solid phase chemical synthesis or by recombinant techniques, although the former is typically more common.

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also be added enzymatically by incorporation into a fusion protein of a *E. coli* BirA substrate peptide.)

The FMOC and tBOC derivatives of dabcyL-L-lysine (Molecular Probes, Inc., Eugene, OR, USA) can be used to incorporate the dabcyL chromophore at selected sites in the peptide sequence during synthesis. The aminonaphthalene derivative EDANS, the most common fluorophore for pairing with the dabcyL quencher in fluorescence resonance energy transfer (FRET) systems, can be introduced during automated synthesis of peptides by using EDANS--FMOC-L-glutamic acid or the corresponding tBOC derivative (both from Molecular Probes, Inc., Eugene, OR, USA). Tetramethylrhodamine fluorophores can be incorporated during automated FMOC synthesis of peptides using (FMOC)--TMR-L-lysine (Molecular Probes, Inc. Eugene, OR, USA).

Other useful amino acid analogues that can be incorporated during chemical synthesis include aspartic acid, glutamic acid, lysine, and tyrosine analogues having allyl side-chain protection (Applied Biosystems, Inc., Foster City, CA, USA); the allyl side chain permits synthesis of cyclic, branched-chain, sulfonated, glycosylated, and phosphorylated peptides.

A large number of other FMOC-protected non-natural amino acid analogues capable of incorporation during chemical synthesis are available commercially, including, e.g., Fmoc-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid, Fmoc-3-endo-aminobicyclo[2.2.1]heptane-2-endo-carboxylic acid, Fmoc-3-exo-aminobicyclo[2.2.1]heptane-2-exo-carboxylic acid, Fmoc-3-endo-amino-bicyclo[2.2.1]hept-5-ene-2-endo-carboxylic acid, Fmoc-3-exo-amino-bicyclo[2.2.1]hept-5-ene-2-exo-carboxylic acid, Fmoc-cis-2-amino-1-cyclohexanecarboxylic acid, Fmoc-trans-2-amino-1-cyclohexanecarboxylic acid, Fmoc-1-amino-1-

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protein gene. When the acylated suppressor tRNA and the mutant gene are combined in an *in vitro* transcription/translation system, the unnatural amino acid is incorporated in response to the UAG codon to give a protein containing that amino acid at the specified position. Liu et al., *Proc. Natl Acad. Sci. USA* 96(9):4780-5 (1999); Wang et al., *Science* 292(5516):498-500 (2001).

The isolated proteins, protein fragments and fusion proteins of the present invention can also include nonnative inter-residue bonds, including bonds that lead to circular and branched forms.

The isolated proteins and protein fragments of the present invention can also include post-translational and post-synthetic modifications, either throughout the length of the protein or localized to one or more portions thereof.

For example, when produced by recombinant expression in eukaryotic cells, the isolated proteins, fragments, and fusion proteins of the present invention will typically include N-linked and/or O-linked glycosylation, the pattern of which will reflect both the availability of glycosylation sites on the protein sequence and the identity of the host cell. Further modification of glycosylation pattern can be performed enzymatically.

As another example, recombinant polypeptides of the invention may also include an initial modified methionine residue, in some cases resulting from host-mediated processes.

When the proteins, protein fragments, and protein fusions of the present invention are produced by chemical synthesis, post-synthetic modification can be performed before deprotection and cleavage from the resin or after deprotection and cleavage. Modification before deprotection

and cleavage of the synthesized protein often allows greater control, e.g. by allowing targeting of the modifying moiety to the N-terminus of a resin-bound synthetic peptide.

Useful post-synthetic (and post-translational)
5 modifications include conjugation to detectable labels, such as fluorophores.

A wide variety of amine-reactive and thiol-reactive fluorophore derivatives have been synthesized that react under nondenaturing conditions with N-terminal amino
10 groups and epsilon amino groups of lysine residues, on the one hand, and with free thiol groups of cysteine residues, on the other.

Kits are available commercially that permit conjugation of proteins to a variety of amine-reactive or
15 thiol-reactive fluorophores: Molecular Probes, Inc. (Eugene, OR, USA), e.g., offers kits for conjugating proteins to Alexa Fluor 350, Alexa Fluor 430, Fluorescein-EX, Alexa Fluor 488, Oregon Green 488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, and
20 Texas Red-X.

A wide variety of other amine-reactive and thiol-reactive fluorophores are available commercially (Molecular Probes, Inc., Eugene, OR, USA), including Alexa Fluor® 350, Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 546, Alexa
25 Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 647 (monoclonal antibody labeling kits available from Molecular Probes, Inc., Eugene, OR, USA), BODIPY dyes, such as BODIPY 493/503, BODIPY FL, BODIPY R6G, BODIPY 530/550, BODIPY TMR, BODIPY 558/568, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589,
30 BODIPY 581/591, BODIPY TR, BODIPY 630/650, BODIPY 650/665, Cascade Blue, Cascade Yellow, Dansyl, lissamine rhodamine B, Marina Blue, Oregon Green 488, Oregon Green 514, Pacific Blue, rhodamine 6G, rhodamine green, rhodamine red,

tetramethylrhodamine, Texas Red (available from Molecular Probes, Inc., Eugene, OR, USA).

The polypeptides of the present invention can also be conjugated to fluorophores, other proteins, and other
5 macromolecules, using bifunctional linking reagents.

Common homobifunctional reagents include, e.g., APG, AEDP, BASED, BMB, BMDB, BMH, BMOE, BM[PEO]3, BM[PEO]4, BS3, BSOCOES, DFDNB, DMA, DMP, DMS, DPDPB, DSG, DSP (Lomant's Reagent), DSS, DST, DTBP, DTME, DTSSP, EGS, HBVS,
10 Sulfo-BSOCOES, Sulfo-DST, Sulfo-EGS (all available from Pierce, Rockford, IL, USA); common heterobifunctional cross-linkers include ABH, AMAS, ANB-NOS, APDP, ASBA, BMPA, BMPH, BMPS, EDC, EMCA, EMCH, EMCS, KMUA, KMUH, GMBS, LC-SMCC, LC-SPDP, MBS, M2C2H, MPBH, MSA, NHS-ASA, PDPH,
15 PMPI, SADP, SAED, SAND, SANPAH, SASD, SATP, SBAP, SFAD, SIA, SIAB, SMCC, SMPB, SMPH, SMPT, SPDP, Sulfo-EMCS, Sulfo-GMBS, Sulfo-HSAB, Sulfo-KMUS, Sulfo-LC-SPDP, Sulfo-MBS, Sulfo-NHS-LC-ASA, Sulfo-SADP, Sulfo-SANPAH, Sulfo-SIAB, Sulfo-SMCC, Sulfo-SMPB, Sulfo-LC-SMPT, SVSB, TFCS (all
20 available Pierce, Rockford, IL, USA).

The proteins, protein fragments, and protein fusions of the present invention can be conjugated, using such cross-linking reagents, to fluorophores that are not amine- or thiol-reactive.

25 Other labels that usefully can be conjugated to the proteins, protein fragments, and fusion proteins of the present invention include radioactive labels, echosonographic contrast reagents, and MRI contrast agents.

The proteins, protein fragments, and protein
30 fusions of the present invention can also usefully be conjugated using cross-linking agents to carrier proteins, such as KLH, bovine thyroglobulin, and even bovine serum

albumin (BSA), to increase immunogenicity for raising anti-MDZ3, anti-MDZ4, anti-MDZ7 or anti-MDZ12 antibodies.

The proteins, protein fragments, and protein fusions of the present invention can also usefully be
5 conjugated to polyethylene glycol (PEG); PEGylation increases the serum half life of proteins administered intravenously for replacement therapy. Delgado et al., *Crit. Rev. Ther. Drug Carrier Syst.* 9(3-4):249-304 (1992); Scott et al., *Curr. Pharm. Des.* 4(6):423-38 (1998);
10 DeSantis et al., *Curr. Opin. Biotechnol.* 10(4):324-30 (1999), incorporated herein by reference in their entirety. PEG monomers can be attached to the protein directly or through a linker, with PEGylation using PEG monomers activated with tresyl chloride
15 (2,2,2-trifluoroethanesulphonyl chloride) permitting direct attachment under mild conditions.

The isolated proteins of the present invention, including fusions thereof, can be produced by recombinant expression, typically using the expression vectors of the
20 present invention as above-described or, if fewer than about 100 amino acids, by chemical synthesis (typically, solid phase synthesis), and, on occasion, by *in vitro* translation.

Production of the isolated proteins of the present invention can optionally be followed by purification.

25 Purification of recombinantly expressed proteins is now well within the skill in the art. See, e.g., Thorner et al. (eds.), Applications of Chimeric Genes and Hybrid Proteins, Part A: Gene Expression and Protein Purification (Methods in Enzymology, Volume 326), Academic Press (2000),
30 (ISBN: 0121822273); Harbin (ed.), Cloning, Gene Expression and Protein Purification : Experimental Procedures and Process Rationale, Oxford Univ. Press (2001) (ISBN: 0195132947); Marshak et al., Strategies for Protein

Purification and Characterization: A Laboratory Course Manual, Cold Spring Harbor Laboratory Press (1996) (ISBN: 0-87969-385-1); and Roe (ed.), Protein Purification Applications, Oxford University Press (2001), the

5 disclosures of which are incorporated herein by reference in their entireties, and thus need not be detailed here.

Briefly, however, if purification tags have been fused through use of an expression vector that appends such tag, purification can be effected, at least in part, by
10 means appropriate to the tag, such as use of immobilized metal affinity chromatography for polyhistidine tags. Other techniques common in the art include ammonium sulfate fractionation, immunoprecipitation, fast protein liquid chromatography (FPLC), high performance liquid
15 chromatography (HPLC), and preparative gel electrophoresis.

Purification of chemically-synthesized peptides can readily be effected, e.g., by HPLC.

Accordingly, it is an aspect of the present invention to provide the isolated proteins of the present
20 invention in pure or substantially pure form.

A purified protein of the present invention is an isolated protein, as above described, that is present at a concentration of at least 95%, as measured on a weight basis (w/w) with respect to total protein in a composition. Such
25 purities can often be obtained during chemical synthesis without further purification, as, e.g., by HPLC. Purified proteins of the present invention can be present at a concentration (measured on a weight basis with respect to total protein in a composition) of 96%, 97%, 98%, and even
30 99%. The proteins of the present invention can even be present at levels of 99.5%, 99.6%, and even 99.7%, 99.8%, or even 99.9% following purification, as by HPLC.

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Although high levels of purity are particularly useful when the isolated proteins of the present invention are used as therapeutic agents - such as vaccines, or for replacement therapy - the isolated proteins of the present invention are also useful at lower purity. For example, partially purified proteins of the present invention can be used as immunogens to raise antibodies in laboratory animals.

Thus, in another aspect, the present invention provides the isolated proteins of the present invention in substantially purified form. A "substantially purified protein" of the present invention is an isolated protein, as above described, present at a concentration of at least 70%, measured on a weight basis with respect to total protein in a composition. Usefully, the substantially purified protein is present at a concentration, measured on a weight basis with respect to total protein in a composition, of at least 75%, 80%, or even at least 85%, 90%, 91%, 92%, 93%, 94%, 94.5% or even at least 94.9%.

In preferred embodiments, the purified and substantially purified proteins of the present invention are in compositions that lack detectable ampholytes, acrylamide monomers, bis-acrylamide monomers, and polyacrylamide.

The proteins, fragments, and fusions of the present invention can usefully be attached to a substrate. The substrate can porous or solid, planar or non-planar; the bond can be covalent or noncovalent.

For example, the proteins, fragments, and fusions of the present invention can usefully be bound to a porous substrate, commonly a membrane, typically comprising nitrocellulose, polyvinylidene fluoride (PVDF), or cationically derivatized, hydrophilic PVDF; so bound, the proteins, fragments, and fusions of the present invention

can be used to detect and quantify antibodies, e.g. in serum, that bind specifically to the immobilized protein of the present invention.

As another example, the proteins, fragments, and fusions of the present invention can usefully be bound to a substantially nonporous substrate, such as plastic, to detect and quantify antibodies, e.g. in serum, that bind specifically to the immobilized protein of the present invention. Such plastics include polymethylacrylic, polyethylene, polypropylene, polyacrylate, polymethylmethacrylate, polyvinylchloride, polytetrafluoroethylene, polystyrene, polycarbonate, polyacetal, polysulfone, celluloseacetate, cellulosenitrate, nitrocellulose, or mixtures thereof; when the assay is performed in standard microtiter dish, the plastic is typically polystyrene.

The proteins, fragments, and fusions of the present invention can also be attached to a substrate suitable for use as a surface enhanced laser desorption ionization source; so attached, the protein, fragment, or fusion of the present invention is useful for binding and then detecting secondary proteins that bind with sufficient affinity or avidity to the surface-bound protein to indicate biologic interaction therebetween. The proteins, fragments, and fusions of the present invention can also be attached to a substrate suitable for use in surface plasmon resonance detection; so attached, the protein, fragment, or fusion of the present invention is useful for binding and then detecting secondary proteins that bind with sufficient affinity or avidity to the surface-bound protein to indicate biological interaction therebetween.

MDZ3 Proteins

In a first series of protein embodiments, the invention provides an isolated MDZ3 polypeptide having an amino acid sequence encoded by the cDNA in ATCC Deposit No. _____, or the amino acid sequence in SEQ ID NO: 3, which are full length MDZ3 proteins. When used as immunogens, the full length proteins of the present invention can be used, *inter alia*, to elicit antibodies that bind to a variety of epitopes of the MDZ3 protein.

The invention further provides fragments of the above-described polypeptides, particularly fragments having at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the sequence given in SEQ ID NO: 3.

The invention further provides fragments of at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the sequence given in SEQ ID NO: 7.

As described above, the invention further provides proteins that differ in sequence from those described with particularity in the above-referenced SEQ ID NOs, whether by way of insertion or deletion, by way of conservative or moderately conservative substitutions, as hybridization related proteins, or as cross-hybridizing proteins.

Particularly useful among the above-described proteins are those having at least one C2H2 (Kruppel family) zinc finger, and especially those that have a plurality of C2H2 zinc fingers in tandem, particularly those that have 7 tandem C2H2 zinc fingers. Also particularly useful among the above-described fragments are those having a SCAN domain, those that encode a KRAB domain, and those that

include all of a SCAN domain, KRAB domain, and 7 zinc fingers.

Also particularly useful are those proteins that have sequence-specific nucleic acid binding regulatory activity, and that participates in protein-protein interactions with other transcription modulators.

The invention further provides fusions of the proteins and protein fragments herein described to heterologous polypeptides.

MDZ4 Proteins

In a first series of protein embodiments, the invention provides an isolated MDZ4 polypeptide having an amino acid sequence encoded by the cDNA in ATCC Deposit No. _____, or the amino acid sequence in SEQ ID NO: 3029, which are full length MDZ4 proteins. When used as immunogens, the full length proteins of the present invention can be used, *inter alia*, to elicit antibodies that bind to a variety of epitopes of the MDZ4 protein.

The invention further provides fragments of the above-described polypeptides, particularly fragments having at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the sequence given in SEQ ID NO: 3029.

The invention further provides fragments of at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the sequence given in SEQ ID Nos: 3033 and 3037.

As described above, the invention further provides proteins that differ in sequence from those described with particularity in the above-referenced SEQ ID NOs., whether by way of insertion or deletion, by way of conservative or

moderately conservative substitutions, as hybridization related proteins, or as cross-hybridizing proteins.

Particularly useful among such proteins are those that have at least one C2H2 (Kruppel family) zinc finger, and especially those that have 5 C2H2 zinc fingers in tandem, those that have a SCAN domain, and those that include all of a SCAN domain and 5 zinc fingers.

Also particularly useful among the above-described MDZ4 proteins are those that have sequence-specific nucleic acid binding regulatory activity, and that participate in protein-protein interactions with other transcription modulators.

The invention further provides fusions of the proteins and protein fragments herein described to heterologous polypeptides.

MDZ7 Proteins

In a first series of protein embodiments, the invention provides an isolated MDZ7 polypeptide having an amino acid sequence encoded by the cDNA in ATCC Deposit No. _____, or the amino acid sequence in SEQ ID NO: 4409, which are full length MDZ7 proteins. When used as immunogens, the full length proteins of the present invention can be used, *inter alia*, to elicit antibodies that bind to a variety of epitopes of the MDZ7 protein.

The invention further provides fragments of the above-described polypeptides, particularly fragments having at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the sequence given in SEQ ID NO: 4409.

As described above, the invention further provides proteins that differ in sequence from those described with

particularity in the above-referenced SEQ ID NOs, whether by way of insertion or deletion, by way of conservative or moderately conservative substitutions, as hybridization related proteins, or as cross-hybridizing proteins, with
5 those that substantially retain a MDZ7 activity particularly useful.

Particularly useful among the above-described MDZ7 proteins are those that have at least one C2H2 (Kruppel family) zinc finger, especially those having a plurality of
10 zinc fingers in tandem, particularly those having 7 zinc fingers in tandem.

Also particularly useful among the above-described MDZ7 proteins are those that have sequence-specific nucleic acid binding regulatory activity, and that function in
15 sequence-specific modulation of gene expression.

The invention further provides fusions of the proteins and protein fragments herein described to heterologous polypeptides.

20 MDZ12 Proteins

In a first series of protein embodiments, the invention provides an isolated MDZ12a polypeptide having an amino acid sequence encoded by the cDNA in ATCC Deposit No.
25 _____, or the amino acid sequence in SEQ ID NO: 5772, which are full length MDZ12a proteins. When used as immunogens, the full length proteins of the present invention can be used, *inter alia*, to elicit antibodies that bind to a variety of epitopes of the MDZ12a protein.

30 The invention further provides fragments of the above-described polypeptides, particularly fragments having at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the

sequence given in SEQ ID NO: 5772.

The invention further provides fragments of at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the
5 sequence given in SEQ ID NO: 5774.

In another series of protein embodiments, the invention provides an isolated MDZ12bS polypeptide having an amino acid sequence encoded by the MDZ12bS part of the MDZ12b cDNA in ATCC Deposit No. _____, or the amino
10 acid sequence in SEQ ID NO: 6939, a full length MDZ12bS protein. When used as immunogens, the full length proteins of the present invention can be used, *inter alia*, to elicit antibodies that bind to a variety of epitopes of the MDZ12bS protein.

The invention further provides fragments of the above-described polypeptides, particularly fragments having at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the
15 sequence given in SEQ ID NO: 6939.

In another series of protein embodiments, the invention provides an isolated MDZ12bL polypeptide having an amino acid sequence encoded by the MDZ12bL portion of the MDZ12b cDNA in ATCC Deposit No. _____, or the amino acid sequence in SEQ ID NO: 6940, which is a full
20 length MDZ12bL protein. When used as immunogens, the full length proteins of the present invention can be used, *inter alia*, to elicit antibodies that bind to a variety of epitopes of the MDZ12bL protein.

The invention further provides fragments of the above-described polypeptides, particularly fragments having at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the
30 sequence given in SEQ ID NO: 6940.

The invention further provides fragments of at least 6 amino acids, typically at least 8 amino acids, often at least 15 amino acids, and even the entirety of the sequence given in SEQ ID NO: 6942.

5 As described above, the invention further provides proteins that differ in sequence from those described with particularity in the above-referenced SEQ ID NOs., whether by way of insertion or deletion, by way of conservative or moderately conservative substitutions, as hybridization
10 related proteins, or as cross-hybridizing proteins, with those that substantially retain a MDZ12 activity particularly useful.

Particularly useful among the above-described MDZ12 proteins are those that have a C2H2 (Kruppel family)
15 zinc finger, particularly those having a plurality of such zinc fingers in tandem, especially those having at least 5, often at least 12, zinc fingers in tandem. Also particularly useful among the above-described proteins are those that have a KRAB-B domain, especially those having
20 both a KRAB domain and at least one, preferably a plurality, especially at least 10, often at least 12, zinc finger domains.

Particularly useful proteins are those that act as sequence-specific transcription regulators, and that
25 interaction with other transcriptional modulators by protein-protein interactions.

The invention further provides fusions of the proteins and protein fragments herein described to heterologous polypeptides.

ANTIBODIES AND ANTIBODY-PRODUCING CELLS

In another aspect, the invention provides antibodies, including fragments and derivatives thereof, that bind specifically to MDZ3, MDZ4, MDZ7 or MDZ12 proteins and protein fragments of the present invention or to one or more of the proteins and protein fragments encoded by the isolated MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids of the present invention. The antibodies of the present invention can be specific for all of linear epitopes, discontinuous epitopes, or conformational epitopes of such proteins or protein fragments, either as present on the protein in its native conformation or, in some cases, as present on the proteins as denatured, as, e.g., by solubilization in SDS.

In other embodiments, the invention provides antibodies, including fragments and derivatives thereof, the binding of which can be competitively inhibited by one or more of the MDZ3, MDZ4, MDZ7 or MDZ12 proteins and protein fragments of the present invention, or by one or more of the proteins and protein fragments encoded by the isolated MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids of the present invention.

As used herein, the term "antibody" refers to a polypeptide, at least a portion of which is encoded by at least one immunoglobulin gene, which can bind specifically to a first molecular species, and to fragments or derivatives thereof that remain capable of such specific binding.

By "bind specifically" and "specific binding" is here intended the ability of the antibody to bind to a first molecular species in preference to binding to other molecular species with which the antibody and first molecular species are admixed. An antibody is said

specifically to "recognize" a first molecular species when it can bind specifically to that first molecular species.

As is well known in the art, the degree to which an antibody can discriminate as among molecular species in a mixture will depend, in part, upon the conformational relatedness of the species in the mixture; typically, the antibodies of the present invention will discriminate over adventitious binding to non-MDZ3, non-MDZ4, non-MDZ7 or non-MDZ12 proteins by at least two-fold, more typically by at least 5-fold, typically by more than 10-fold, 25-fold, 50-fold, 75-fold, and often by more than 100-fold, and on occasion by more than 500-fold or 1000-fold. When used to detect the proteins or protein fragments of the present invention, the antibody of the present invention is sufficiently specific when it can be used to determine the presence of the protein of the present invention in samples derived from human tissues expressing each of the genes (see, e.g., Examples 1, 2, 3, and 4).

Typically, the affinity or avidity of an antibody (or antibody multimer, as in the case of an IgM pentamer) of the present invention for a protein or protein fragment of the present invention will be at least about 1×10^{-6} molar (M), typically at least about 5×10^{-7} M, usefully at least about 1×10^{-7} M, with affinities and avidities of at least 1×10^{-8} M, 5×10^{-9} M, and 1×10^{-10} M proving especially useful.

The antibodies of the present invention can be naturally-occurring forms, such as IgG, IgM, IgD, IgE, and IgA, from any mammalian species.

Human antibodies can, but will infrequently, be drawn directly from human donors or human cells. In such case, antibodies to the proteins of the present invention will typically have resulted from fortuitous immunization,

such as autoimmune immunization, with the protein or protein fragments of the present invention. Such antibodies will typically, but will not invariably, be polyclonal.

Human antibodies are more frequently obtained
5 using transgenic animals that express human immunoglobulin genes, which transgenic animals can be affirmatively immunized with the protein immunogen of the present invention. Human Ig-transgenic mice capable of producing human antibodies and methods of producing human antibodies
10 therefrom upon specific immunization are described, *inter alia*, in U.S. Patent Nos. 6,162,963; 6,150,584; 6,114,598; 6,075,181; 5,939,598; 5,877,397; 5,874,299; 5,814,318; 5,789,650; 5,770,429; 5,661,016; 5,633,425; 5,625,126; 5,569,825; 5,545,807; 5,545,806, and 5,591,669, the
15 disclosures of which are incorporated herein by reference in their entirety. Such antibodies are typically monoclonal, and are typically produced using techniques developed for production of murine antibodies.

Human antibodies are particularly useful, and
20 often preferred, when the antibodies of the present invention are to be administered to human beings as *in vivo* diagnostic or therapeutic agents, since recipient immune response to the administered antibody will often be substantially less than that occasioned by administration of
25 an antibody derived from another species, such as mouse.

IgG, IgM, IgD, IgE and IgA antibodies of the present invention are also usefully obtained from other mammalian species, including rodents - typically mouse, but also rat, guinea pig, and hamster - lagomorphs, typically
30 rabbits, and also larger mammals, such as sheep, goats, cows, and horses. In such cases, as with the transgenic human-antibody-producing non-human mammals, fortuitous immunization is not required, and the non-human mammal is

typically affirmatively immunized, according to standard immunization protocols, with the protein or protein fragment of the present invention.

As discussed above, virtually all fragments of 8
5 or more contiguous amino acids of the proteins of the present invention can be used effectively as immunogens when conjugated to a carrier, typically a protein such as bovine thyroglobulin, keyhole limpet hemocyanin, or bovine serum albumin, conveniently using a bifunctional linker such as
10 those described elsewhere above, which discussion is incorporated by reference here.

Immunogenicity can also be conferred by fusion of the proteins and protein fragments of the present invention to other moieties.

15 For example, peptides of the present invention can be produced by solid phase synthesis on a branched polylysine core matrix; these multiple antigenic peptides (MAPs) provide high purity, increased avidity, accurate chemical definition and improved safety in vaccine
20 development. Tam *et al.*, Proc. Natl. Acad. Sci. USA 85:5409-5413 (1988); Posnett *et al.*, *J. Biol. Chem.* 263, 1719-1725 (1988).

Protocols for immunizing non-human mammals are well-established in the art, Harlow *et al.* (eds.),
25 Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory (1998) (ISBN: 0879693142); Coligan *et al.* (eds.), Current Protocols in Immunology, John Wiley & Sons, Inc. (2001) (ISBN: 0-471-52276-7); Zola, Monoclonal Antibodies : Preparation and Use of Monoclonal Antibodies and Engineered
30 Antibody Derivatives (Basics: From Background to Bench), Springer Verlag (2000) (ISBN: 0387915907), the disclosures of which are incorporated herein by reference, and often include multiple immunizations, either with or without

Antibodies from nonhuman mammals can be polyclonal or monoclonal, with polyclonal antibodies having certain advantages in immunohistochemical detection of the proteins of the present invention and monoclonal antibodies having advantages in identifying and distinguishing particular epitopes of the proteins of the present invention.

Briefly, however, such techniques include, *inter alia*, production of monoclonal antibodies by hybridomas and expression of antibodies or fragments or derivatives thereof from host cells engineered to express immunoglobulin genes or fragments thereof. These two methods of production are not mutually exclusive: genes encoding antibodies specific

for the proteins or protein fragments of the present invention can be cloned from hybridomas and thereafter expressed in other host cells. Nor need the two necessarily be performed together: e.g., genes encoding antibodies
5 specific for the proteins and protein fragments of the present invention can be cloned directly from B cells known to be specific for the desired protein, as further described in U.S. Pat. No. 5,627,052, the disclosure of which is incorporated herein by reference in its entirety, or from
10 antibody-displaying phage.

Recombinant expression in host cells is particularly useful when fragments or derivatives of the antibodies of the present invention are desired.

Host cells for recombinant antibody production -
15 either whole antibodies, antibody fragments, or antibody derivatives - can be prokaryotic or eukaryotic.

Prokaryotic hosts are particularly useful for producing phage displayed antibodies of the present invention.

20 The technology of phage-displayed antibodies, in which antibody variable region fragments are fused, for example, to the gene III protein (pIII) or gene VIII protein (pVIII) for display on the surface of filamentous phage, such as M13, is by now well-established, Sidhu, *Curr. Opin. Biotechnol.* 11(6):610-6 (2000); Griffiths *et al.*, *Curr. Opin. Biotechnol.* 9(1):102-8 (1998); Hoogenboom *et al.*, *Immunotechnology*, 4(1):1-20 (1998); Rader *et al.*, *Current Opinion in Biotechnology* 8:503-508 (1997); Aujame *et al.*, *Human Antibodies* 8:155-168 (1997); Hoogenboom, *Trends in Biotechnol.* 15:62-70 (1997); de Kruif *et al.*, 17:453-455 (1996); Barbas *et al.*, *Trends in Biotechnol.* 14:230-234 (1996); Winter *et al.*, *Ann. Rev. Immunol.* 433-455 (1994),
30 and techniques and protocols required to generate,

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propagate, screen (pan), and use the antibody fragments from such libraries have recently been compiled, Barbas et al., Phage Display: A Laboratory Manual, Cold Spring Harbor Laboratory Press (2001) (ISBN 0-87969-546-3); Kay et al.

5 (eds.), Phage Display of Peptides and Proteins: A Laboratory Manual, Academic Press, Inc. (1996); Abelson et al. (eds.), Combinatorial Chemistry, Methods in Enzymology vol. 267, Academic Press (May 1996), the disclosures of which are incorporated herein by reference in their entireties.

10 Typically, phage-displayed antibody fragments are scFv fragments or Fab fragments; when desired, full length antibodies can be produced by cloning the variable regions from the displaying phage into a complete antibody and expressing the full length antibody in a further prokaryotic
15 or a eukaryotic host cell.

Eukaryotic cells are also useful for expression of the antibodies, antibody fragments, and antibody derivatives of the present invention.

For example, antibody fragments of the present
20 invention can be produced in *Pichia pastoris*, Takahashi et al., *Biosci. Biotechnol. Biochem.* 64(10):2138-44 (2000); Freyre et al., *J. Biotechnol.* 76(2-3):157-63 (2000); Fischer et al., *Biotechnol. Appl. Biochem.* 30 (Pt 2):117-20 (1999); Pennell et al., *Res. Immunol.* 149(6):599-603 (1998); Eldin
25 et al., *J. Immunol. Methods.* 201(1):67-75 (1997); and in *Saccharomyces cerevisiae*, Frenken et al., *Res. Immunol.* 149(6):589-99 (1998); Shusta et al., *Nature Biotechnol.* 16(8):773-7 (1998), the disclosures of which are incorporated herein by reference in their entireties.

30 Antibodies, including antibody fragments and derivatives, of the present invention can also be produced in insect cells, Li et al., *Protein Expr. Purif.* 21(1):121-8 (2001); Ailor et al., *Biotechnol. Bioeng.* 58(2-3):196-203

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(1998); Hsu et al., *Biotechnol. Prog.* 13(1):96-104 (1997); Edelman et al., *Immunology* 91(1):13-9 (1997); and Nesbit et al., *J. Immunol. Methods*. 151(1-2):201-8 (1992), the disclosures of which are incorporated herein by reference in their entireties.

Antibodies and fragments and derivatives thereof of the present invention can also be produced in plant cells, Giddings et al., *Nature Biotechnol.* 18(11):1151-5 (2000); Gavilondo et al., *Biotechniques* 29(1):128-38 (2000); Fischer et al., *J. Biol. Regul. Homeost. Agents* 14(2):83-92 (2000); Fischer et al., *Biotechnol. Appl. Biochem.* 30 (Pt 2):113-6 (1999); Fischer et al., *Biol. Chem.* 380(7-8):825-39 (1999); Russell, *Curr. Top. Microbiol. Immunol.* 240:119-38 (1999); and Ma et al., *Plant Physiol.* 109(2):341-6 (1995), the disclosures of which are incorporated herein by reference in their entireties.

Mammalian cells useful for recombinant expression of antibodies, antibody fragments, and antibody derivatives of the present invention include CHO cells, COS cells, 293 cells, and myeloma cells.

Verma et al., *J. Immunol. Methods* 216(1-2):165-81 (1998), review and compare bacterial, yeast, insect and mammalian expression systems for expression of antibodies.

Antibodies of the present invention can also be prepared by cell free translation, as further described in Merk et al., *J. Biochem. (Tokyo)*. 125(2):328-33 (1999) and Ryabova et al., *Nature Biotechnol.* 15(1):79-84 (1997), and in the milk of transgenic animals, as further described in Pollock et al., *J. Immunol. Methods* 231(1-2):147-57 (1999), the disclosures of which are incorporated herein by reference in their entireties.

The invention further provides antibody fragments that bind specifically to one or more of the proteins and

protein fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention.

Among such useful fragments are Fab, Fab', Fv, F(ab)'₂, and single chain Fv (scFv) fragments. Other useful fragments are described in Hudson, *Curr. Opin. Biotechnol.* 9(4):395-402 (1998).

It is also an aspect of the present invention to provide antibody derivatives that bind specifically to one or more of the proteins and protein fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention.

Among such useful derivatives are chimeric, primatized, and humanized antibodies; such derivatives are less immunogenic in human beings, and thus more suitable for in vivo administration, than are unmodified antibodies from non-human mammalian species.

Chimeric antibodies typically include heavy and/or light chain variable regions (including both CDR and framework residues) of immunoglobulins of one species, typically mouse, fused to constant regions of another species, typically human. See, e.g., U.S. Pat. No. 5,807,715; Morrison et al., *Proc. Natl. Acad. Sci*

1.032990" 1822550

USA.81(21):6851-5 (1984); Sharon et al., *Nature*
309(5966):364-7 (1984); Takeda et al., *Nature*
314(6010):452-4 (1985), the disclosures of which are
incorporated herein by reference in their entireties.

5 Primatized and humanized antibodies typically include heavy
and/or light chain CDRs from a murine antibody grafted into
a non-human primate or human antibody V region framework,
usually further comprising a human constant region,
Riechmann et al., *Nature* 332(6162):323-7 (1988); Co et al.,
10 *Nature* 351(6326):501-2 (1991); U.S. Pat. Nos. 6,054,297;
5,821,337; 5,770,196; 5,766,886; 5,821,123; 5,869,619;
6,180,377; 6,013,256; 5,693,761; and 6,180,370, the
disclosures of which are incorporated herein by reference in
their entireties.

15 Other useful antibody derivatives of the invention include heteromeric antibody complexes and antibody fusions, such as diabodies (bisppecific antibodies), single-chain diabodies, and intrabodies.

The antibodies of the present invention, including
20 fragments and derivatives thereof, can usefully be labeled.

It is, therefore, another aspect of the present invention to provide labeled antibodies that bind specifically to one or more of the proteins and protein fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention.

The choice of label depends, in part, upon the desired use.

For example, when the antibodies of the present invention are used for immunohistochemical staining of tissue samples, the label can usefully be an enzyme that catalyzes production and local deposition of a detectable product.

Enzymes typically conjugated to antibodies to permit their immunohistochemical visualization are well known, and include alkaline phosphatase, β -galactosidase, glucose oxidase, horseradish peroxidase (HRP), and urease. Typical substrates for production and deposition of visually detectable products include o-nitrophenyl-beta-D-galactopyranoside (ONPG); o-phenylenediamine dihydrochloride (OPD); p-nitrophenyl phosphate (PNPP); p-nitrophenyl-beta-D-galactopyranoside (PNPG); 3',3'-diaminobenzidine (DAB); 3-amino-9-ethylcarbazole (AEC); 4-chloro-1-naphthol (CN); 5-bromo-4-chloro-3-indolyl-phosphate (BCIP); ABTS®; BluoGal; iodonitrotetrazolium (INT); nitroblue tetrazolium chloride (NBT); phenazine methosulfate (PMS); phenolphthalein monophosphate (PMP); tetramethyl benzidine (TMB); tetranitroblue tetrazolium (TNBT); X-Gal; X-Gluc; and X-Glucoside.

Other substrates can be used to produce products for local deposition that are luminescent. For example, in the presence of hydrogen peroxide (H_2O_2), horseradish peroxidase (HRP) can catalyze the oxidation of cyclic diacylhydrazides, such as luminol. Immediately following the oxidation, the luminol is in an excited state (intermediate reaction product), which decays to the ground state by emitting light. Strong enhancement of the light emission is produced by enhancers, such as phenolic compounds. Advantages include high sensitivity, high resolution, and rapid detection without radioactivity and

requiring only small amounts of antibody. See, e.g., Thorpe
et al., *Methods Enzymol.* 133:331-53 (1986); Kricka et al.,
J. Immunoassay 17(1):67-83 (1996); and Lundqvist et al., *J.*
Biolumin. Chemilumin. 10(6):353-9 (1995), the disclosures of
5 which are incorporated herein by reference in their
entireties. Kits for such enhanced chemiluminescent
detection (ECL) are available commercially.

The antibodies can also be labeled using colloidal
gold.

10 As another example, when the antibodies of the
present invention are used, e.g., for flow cytometric
detection, for scanning laser cytometric detection, or for
fluorescent immunoassay, they can usefully be labeled with
fluorophores.

15 There are a wide variety of fluorophore labels
that can usefully be attached to the antibodies of the
present invention.

For flow cytometric applications, both for
extracellular detection and for intracellular detection,
20 common useful fluorophores can be fluorescein isothiocyanate
(FITC), allophycocyanin (APC), R-phycoerythrin (PE),
peridinin chlorophyll protein (PerCP), Texas Red, Cy3, Cy5,
fluorescence resonance energy tandem fluorophores such as
PerCP-Cy5.5, PE-Cy5, PE-Cy5.5, PE-Cy7, PE-Texas Red, and
25 APC-Cy7.

Other fluorophores include, *inter alia*, Alexa
Fluor® 350, Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor®
546, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 647
(monoclonal antibody labeling kits available from Molecular
30 Probes, Inc., Eugene, OR, USA), BODIPY dyes, such as BODIPY
493/503, BODIPY FL, BODIPY R6G, BODIPY 530/550, BODIPY TMR,
BODIPY 558/568, BODIPY 558/568, BODIPY 564/570, BODIPY
576/589, BODIPY 581/591, BODIPY TR, BODIPY 630/650, BODIPY

650/665, Cascade Blue, Cascade Yellow, Dansyl, lissamine
rhodamine B, Marina Blue, Oregon Green 488, Oregon Green
514, Pacific Blue, rhodamine 6G, rhodamine green, rhodamine
red, tetramethylrhodamine, Texas Red (available from
5 Molecular Probes, Inc., Eugene, OR, USA), and Cy2, Cy3,
Cy3.5, Cy5, Cy5.5, Cy7, all of which are also useful for
fluorescently labeling the antibodies of the present
invention.

For secondary detection using labeled avidin,
10 streptavidin, captavidin or neutravidin, the antibodies of
the present invention can usefully be labeled with biotin.

When the antibodies of the present invention are
used, e.g., for western blotting applications, they can
usefully be labeled with radioisotopes, such as ^{33}P , ^{32}P , ^{35}S ,
15 ^3H , and ^{125}I .

As another example, when the antibodies of the
present invention are used for radioimmunotherapy, the label
can usefully be ^{228}Th , ^{227}Ac , ^{225}Ac , ^{223}Ra , ^{213}Bi , ^{212}Pb , ^{212}Bi ,
 ^{211}At , ^{203}Pb , ^{194}Os , ^{188}Re , ^{186}Re , ^{153}Sm , ^{149}Tb , ^{131}I , ^{125}I , ^{111}In ,
20 ^{105}Rh , $^{99\text{m}}\text{Tc}$, ^{97}Ru , ^{90}Y , ^{90}Sr , ^{88}Y , ^{72}Se , ^{67}Cu , or ^{47}Sc .

As another example, when the antibodies of the
present invention are to be used for *in vivo* diagnostic use,
they can be rendered detectable by conjugation to MRI
contrast agents, such as gadolinium
25 diethylenetriaminepentaacetic acid (DTPA), Lauffer et al.,
Radiology 207(2):529-38 (1998), or by radioisotopic labeling

As would be understood, use of the labels
described above is not restricted to the application as for
which they were mentioned.

30 The antibodies of the present invention, including
fragments and derivatives thereof, can also be conjugated to
toxins, in order to target the toxin's ablative action to
cells that display and/or express the proteins of the

present invention. Commonly, the antibody in such immunotoxins is conjugated to Pseudomonas exotoxin A, diphtheria toxin, shiga toxin A, anthrax toxin lethal factor, or ricin. See Hall (ed.), Immunotoxin Methods and
5 Protocols (Methods in Molecular Biology, Vol 166), Humana Press (2000) (ISBN:0896037754); and Frankel et al. (eds.), Clinical Applications of Immunotoxins, Springer-Verlag New York, Incorporated (1998) (ISBN:3540640975), the disclosures of which are incorporated herein by reference in their
10 entireties, for review.

The antibodies of the present invention can usefully be attached to a substrate, and it is, therefore, another aspect of the invention to provide antibodies that bind specifically to one or more of the proteins and protein
15 fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or
20 one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, attached to a substrate.

Substrates can be porous or nonporous, planar or nonplanar.

25 For example, the antibodies of the present invention can usefully be conjugated to filtration media, such as NHS-activated Sepharose or CNBr-activated Sepharose for purposes of immunoaffinity chromatography.

For example, the antibodies of the present
30 invention can usefully be attached to paramagnetic microspheres, typically by biotin-streptavidin interaction, which microsphere can then be used for isolation of cells that express or display the proteins of the present

invention. As another example, the antibodies of the present invention can usefully be attached to the surface of a microtiter plate for ELISA.

As noted above, the antibodies of the present invention can be produced in prokaryotic and eukaryotic cells. It is, therefore, another aspect of the present invention to provide cells that express the antibodies of the present invention, including hybridoma cells, B cells, plasma cells, and host cells recombinantly modified to express the antibodies of the present invention.

In yet a further aspect, the present invention provides aptamers evolved to bind specifically to one or more of the proteins and protein fragments of the present invention, to one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention, or the binding of which can be competitively inhibited by one or more of the proteins and protein fragments of the present invention or one or more of the proteins and protein fragments encoded by the isolated nucleic acids of the present invention.

MDZ3 Antibodies

In a first series of antibody embodiments, the invention provides antibodies, both polyclonal and monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be competitively inhibited by, a polypeptide having an amino acid sequence encoded by the MDZ3 cDNA in ATCC Deposit No. _____, or having the amino acid sequence of SEQ ID No: 3.

In a second series of antibody embodiments, the invention provides antibodies, both polyclonal and

monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be competitively inhibited by, a polypeptide having the amino acid sequence of SEQ ID NO:7.

5 In a third series of antibody embodiments, the invention provides antibodies, both polyclonal and monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be competitively inhibited by, polypeptides encoded by any of
10 the MDZ3 nucleic acids of the present invention, as above-described.

 Such antibodies are useful in *in vitro* immunoassays, such as ELISA, western blot or immunohistochemical assay, for detection of MDZ3 and related
15 proteins. Such antibodies are also useful in isolating and purifying MDZ3 proteins, including related cross-reactive proteins, by immunoprecipitation, immunoaffinity chromatography, or magnetic bead-mediated purification.

 In other embodiments, the invention further
20 provides the above-described antibodies detectably labeled, and in yet other embodiments, provides the above-described antibodies attached to a substrate.

MDZ4 Antibodies

25 In a first series of antibody embodiments, the invention provides antibodies, both polyclonal and monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be
30 competitively inhibited by, a polypeptide having an amino acid sequence encoded by the MDZ4 cDNA in ATCC Deposit No. _____, or having the amino acid sequence of SEQ ID No: 3029.

In a second series of antibody embodiments, the invention provides antibodies, both polyclonal and monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be competitively inhibited by, a polypeptide having the amino acid sequence of SEQ ID NO:3033.

In a third series of antibody embodiments, the invention provides antibodies, both polyclonal and monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be competitively inhibited by, a polypeptide having the amino acid sequence of SEQ ID NO:3037.

In a fourth series of antibody embodiments, the invention provides antibodies, both polyclonal and monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be competitively inhibited by, polypeptides encoded by any of the MDZ7 nucleic acids of the present invention, as above-described.

Such antibodies are useful in *in vitro* immunoassays, such as ELISA, western blot or immunohistochemical assay, for detection of MDZ3 and related proteins. Such antibodies are also useful in isolating and purifying MDZ4 proteins, including related cross-reactive proteins, by immunoprecipitation, immunoaffinity chromatography, or magnetic bead-mediated purification.

In other embodiments, the invention further provides the above-described antibodies detectably labeled, and in yet other embodiments, provides the above-described antibodies attached to a substrate.

MDZ7 Antibodies

In a first series of antibody embodiments of this aspect of the invention, the invention provides antibodies, both polyclonal and monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be competitively inhibited by, a polypeptide having an amino acid sequence encoded by the MDZ7 cDNA in ATCC Deposit No. _____, or having the amino acid sequence of SEQ ID No: 4409.

Such antibodies are useful in *in vitro* immunoassays, such as ELISA, western blot or immunohistochemical assay, for detection of MDZ7 and related proteins. Such antibodies are also useful in isolating and purifying MDZ7 proteins, including related cross-reactive proteins, by immunoprecipitation, immunoaffinity chromatography, or magnetic bead-mediated purification.

In other embodiments, the invention further provides the above-described antibodies detectably labeled, and in yet other embodiments, provides the above-described antibodies attached to a substrate.

MDZ12a, MDZ12bS and MDZ12bL Antibodies

The invention further provides antibodies, polyclonal or monoclonal, and fragments and derivatives thereof, that bind specifically to, or the binding of which can be competitively inhibited by, a polypeptide having an amino acid sequence encoded by the MDZ12a cDNA in ATCC Deposit No. _____, or having the amino acid sequence of SEQ ID No:5772. Depending upon the epitope recognized, certain of such antibodies can cross-react with MDZ12bS, others with MDZ12bL, and yet others with neither

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proteins, by immunoprecipitation, immunoaffinity chromatography, or magnetic bead-mediated purification.

In other embodiments, the invention further provides the above-described antibodies detectably labeled, and in yet other embodiments, provides the above-described antibodies attached to a substrate.

PHARMACEUTICAL COMPOSITIONS

MDZ3, MDZ4, MDZ7 and MDZ12 are important for transcriptional regulation and protein-protein interactions with other transcription modulators; defects in MDZ3, MDZ4, MDZ7 or MDZ12 expression, activity, distribution, localization, and/or solubility are a cause of human disease, which disease can manifest as a disorder of brain, testis, heart or bone marrow function for MDZ3; bone marrow, brain, heart, hela, adult liver, fetal liver, lung, placenta or prostate function for MDZ4; testes function for MDZ7; and brain, heart, kidney, placenta, skeletal muscle, testis, bone marrow or liver function for MDZ12.

Accordingly, pharmaceutical compositions comprising nucleic acids, proteins, and antibodies of the present invention, as well as mimetics, agonists, antagonists, or inhibitors of MDZ3, MDZ4, MDZ7 or MDZ12 activity, can be administered as therapeutics for treatment of MDZ3, MDZ4, MDZ7 or MDZ12 defects, respectively.

Thus, in another aspect, the invention provides pharmaceutical compositions comprising the nucleic acids, nucleic acid fragments, proteins, protein fusions, protein fragments, antibodies, antibody derivatives, antibody fragments, mimetics, agonists, antagonists, and inhibitors of the present invention.

Such a composition typically contains from about 0.1 to 90% by weight of a therapeutic agent of the invention formulated in and/or with a pharmaceutically acceptable carrier or excipient.

5 Pharmaceutical formulation is a well-established art, and is further described in Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20th ed., Lippincott, Williams & Wilkins (2000) (ISBN: 0683306472); Ansel et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th
10 ed., Lippincott Williams & Wilkins Publishers (1999) (ISBN: 0683305727); and Kibbe (ed.), Handbook of Pharmaceutical Excipients American Pharmaceutical Association, 3rd ed. (2000) (ISBN: 091733096X), the disclosures of which are incorporated herein by reference in their entireties, and
15 thus need not be described in detail herein.

Briefly, however, formulation of the pharmaceutical compositions of the present invention will depend upon the route chosen for administration. The pharmaceutical compositions utilized in this invention can
20 be administered by various routes including both enteral and parenteral routes, including oral, intravenous, intramuscular, subcutaneous, inhalation, topical, sublingual, rectal, intra-arterial, intramedullary, intrathecal, intraventricular, transmucosal, transdermal,
25 intranasal, intraperitoneal, intrapulmonary, and intrauterine.

Oral dosage forms can be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

30 Solid formulations of the compositions for oral administration can contain suitable carriers or excipients, such as carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch

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from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, or microcrystalline cellulose; gums including arabic and tragacanth; proteins
5 such as gelatin and collagen; inorganics, such as kaolin, calcium carbonate, dicalcium phosphate, sodium chloride; and other agents such as acacia and alginic acid.

Agents that facilitate disintegration and/or solubilization can be added, such as the cross-linked
10 polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate, microcrystalline cellulose, corn starch, sodium starch glycolate, and alginic acid.

Tablet binders that can be used include acacia,
15 methylcellulose, sodium carboxymethylcellulose, polyvinylpyrrolidone (PovidoneTM), hydroxypropyl methylcellulose, sucrose, starch and ethylcellulose.

Lubricants that can be used include magnesium stearates, stearic acid, silicone fluid, talc, waxes, oils,
20 and colloidal silica.

Fillers, agents that facilitate disintegration and/or solubilization, tablet binders and lubricants, including the aforementioned, can be used singly or in combination.

25 Solid oral dosage forms need not be uniform throughout.

For example, dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which can also contain gum arabic, talc,
30 polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures.

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Oral dosage forms of the present invention include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients
5 mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol
10 with or without stabilizers.

Additionally, dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

15 Liquid formulations of the pharmaceutical compositions for oral (enteral) administration are prepared in water or other aqueous vehicles and can contain various suspending agents such as methylcellulose, alginates, tragacanth, pectin, kelgin, carrageenan, acacia,
20 polyvinylpyrrolidone, and polyvinyl alcohol. The liquid formulations can also include solutions, emulsions, syrups and elixirs containing, together with the active compound(s), wetting agents, sweeteners, and coloring and flavoring agents.

25 The pharmaceutical compositions of the present invention can also be formulated for parenteral administration.

For intravenous injection, water soluble versions of the compounds of the present invention are formulated in,
30 or if provided as a lyophilate, mixed with, a physiologically acceptable fluid vehicle, such as 5% dextrose ("D5"), physiologically buffered saline, 0.9% saline, Hanks' solution, or Ringer's solution.

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Intramuscular preparations, e.g. a sterile formulation of a suitable soluble salt form of the compounds of the present invention, can be dissolved and administered in a pharmaceutical excipient such as Water-for-Injection, 0.9% saline, or 5% glucose solution. Alternatively, a suitable insoluble form of the compound can be prepared and administered as a suspension in an aqueous base or a pharmaceutically acceptable oil base, such as an ester of a long chain fatty acid (e.g., ethyl oleate), fatty oils such as sesame oil, triglycerides, or liposomes.

Parenteral formulations of the compositions can contain various carriers such as vegetable oils, dimethylacetamide, dimethylformamide, ethyl lactate, ethyl carbonate, isopropyl myristate, ethanol, polyols (glycerol, propylene glycol, liquid polyethylene glycol, and the like).

Aqueous injection suspensions can also contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Non-lipid polycationic amino polymers can also be used for delivery. Optionally, the suspension can also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical compositions of the present invention can also be formulated to permit injectable, long-term, deposition.

The pharmaceutical compositions of the present invention can be administered topically.

A topical semi-solid ointment formulation typically contains a concentration of the active ingredient from about 1 to 20%, e.g., 5 to 10%, in a carrier such as a pharmaceutical cream base. Various formulations for topical

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use include drops, tinctures, lotions, creams, solutions, and ointments containing the active ingredient and various supports and vehicles. In other transdermal formulations, typically in patch-delivered formulations, the
5 pharmaceutically active compound is formulated with one or more skin penetrants, such as 2-N-methyl-pyrrolidone (NMP) or Azone.

Inhalation formulations can also readily be formulated. For inhalation, various powder and liquid
10 formulations can be prepared.

The pharmaceutically active compound in the pharmaceutical compositions of the present invention can be provided as the salt of a variety of acids, including but not limited to hydrochloric, sulfuric, acetic, lactic,
15 tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms.

After pharmaceutical compositions have been prepared, they are packaged in an appropriate container and
20 labeled for treatment of an indicated condition.

The active compound will be present in an amount effective to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

25 A "therapeutically effective dose" refers to that amount of active ingredient - for example MDZ3, MDZ4, MDZ7 or MDZ12 protein, fusion protein, or fragments thereof, antibodies specific for MDZ3, MDZ4, MDZ7 or MDZ12, agonists, antagonists or inhibitors of MDZ3, MDZ4, MDZ7 or MDZ12 -
30 which ameliorates the signs or symptoms of the disease or prevents progression thereof; as would be understood in the medical arts, cure, although desired, is not required.

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The therapeutically effective dose of the pharmaceutical agents of the present invention can be estimated initially by *in vitro* tests, such as cell culture assays, followed by assay in model animals, usually mice, rats, rabbits, dogs, or pigs. The animal model can also be used to determine an initial useful concentration range and route of administration.

For example, the ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population) can be determined in one or more cell culture or animal model systems. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as LD50/ED50. Pharmaceutical compositions that exhibit large therapeutic indices are particularly useful.

The data obtained from cell culture assays and animal studies is used in formulating an initial dosage range for human use, and preferably provides a range of circulating concentrations that includes the ED50 with little or no toxicity. After administration, or between successive administrations, the circulating concentration of active agent varies within this range depending upon pharmacokinetic factors well known in the art, such as the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors specific to the subject requiring treatment. Factors that can be taken into account by the practitioner include the severity of the disease state, general health of the subject, age, weight, gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical

compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Where the therapeutic agent is a protein or antibody of the present invention, the therapeutic protein or antibody agent typically is administered at a daily dosage of 0.01 mg to 30 mg/kg of body weight of the patient (e.g., 1mg/kg to 5 mg/kg). The pharmaceutical formulation can be administered in multiple doses per day, if desired, to achieve the total desired daily dose.

Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the pharmaceutical formulation(s) of the present invention to the patient. The pharmaceutical compositions of the present invention can be administered alone, or in combination with other therapeutic agents or interventions.

THERAPEUTIC METHODS

The present invention further provides methods of treating subjects having defects in MDZ3, MDZ4, MDZ7 or MDZ12 - e.g., in expression, activity, distribution, localization, and/or solubility of MDZ3, MDZ4, MDZ7 or MDZ12

- which can manifest as a disorder of brain, testis, heart or bone marrow function for MDZ3; bone marrow, brain, heart, hela, adult liver, fetal liver, lung, placenta and prostate function for MDZ4; testes function for MDZ7; and brain, heart, kidney, placenta, skeletal muscle, testis, bone marrow or liver function for MDZ12. As used herein, "treating" includes all medically-acceptable types of therapeutic intervention, including palliation and prophylaxis (prevention) of disease.

In one embodiment of the therapeutic methods of the present invention, a therapeutically effective amount of a pharmaceutical composition comprising MDZ3, MDZ4, MDZ7 or MDZ12 protein, fusion, fragment or derivative thereof is administered to a subject with a clinically-significant MDZ3, MDZ4, MDZ7 or MDZ12 defect.

Protein compositions are administered, for example, to complement a deficiency in native MDZ3, MDZ4, MDZ7 or MDZ12, respectively. In other embodiments, protein compositions are administered as a vaccine to elicit a humoral and/or cellular immune response to MDZ3, MDZ4, MDZ7 or MDZ12, respectively. The immune response can be used to modulate activity of MDZ3, MDZ4, MDZ7 or MDZ12, respectively, or, depending on the immunogen, to immunize against aberrant or aberrantly expressed forms, such as mutant or inappropriately expressed isoforms. In yet other embodiments, protein fusions having a toxic moiety are administered to ablate cells that aberrantly accumulate MDZ3, MDZ4, MDZ7 or MDZ12, respectively.

In another embodiment of the therapeutic methods of the present invention, a therapeutically effective amount of a pharmaceutical composition comprising nucleic acid of the present invention is administered. The nucleic acid can be delivered in a vector that drives expression of MDZ3,

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MDZ4, MDZ7 or MDZ12 protein, fusion, or fragment thereof, or without such vector.

Nucleic acid compositions that can drive expression of MDZ3, MDZ4, MDZ7 or MDZ12 are administered, for example, to complement a deficiency in native MDZ3, MDZ4, MDZ7 or MDZ12, or as DNA vaccines. Expression vectors derived from virus, replication deficient retroviruses, adenovirus, adeno-associated (AAV) virus, herpes virus, or vaccinia virus can be used - see, e.g., Cid-Arregui (ed.), Viral Vectors: Basic Science and Gene Therapy, Eaton Publishing Co., 2000 (ISBN: 188129935X) - as can plasmids .

Antisense nucleic acid compositions, or vectors that drive expression of MDZ3, MDZ4, MDZ7 or MDZ12 antisense nucleic acids, are administered to downregulate transcription and/or translation of MDZ3, MDZ4, MDZ7 or MDZ12 in circumstances in which excessive production, or production of aberrant protein, is the pathophysiologic basis of disease.

Antisense compositions useful in therapy can have sequence that is complementary to coding or to noncoding regions of the MDZ3, MDZ4, MDZ7 or MDZ12 genes, respectively. For example, oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are particularly useful.

Catalytic antisense compositions, such as ribozymes, that are capable of sequence-specific hybridization to MDZ3, MDZ4, MDZ7 or MDZ12 transcripts, are also useful in therapy. See, e.g., Phylactou, *Adv. Drug Deliv. Rev.* 44(2-3):97-108 (2000); Phylactou et al., *Hum. Mol. Genet.* 7(10):1649-53 (1998); Rossi, *Ciba Found. Symp.* 209:195-204 (1997); and Sigurdsson et al., *Trends Biotechnol.* 13(8):286-9 (1995), the disclosures of which are incorporated herein by reference in their entireties.

Other nucleic acids useful in the therapeutic methods of the present invention are those that are capable of triplex helix formation in or near the MDZ3, MDZ4, MDZ7 or MDZ12 genomic locus, respectively. Such triplexing oligonucleotides are able to inhibit transcription, Intody et al., *Nucleic Acids Res.* 28(21):4283-90 (2000); McGuffie et al., *Cancer Res.* 60(14):3790-9 (2000), the disclosures of which are incorporated herein by reference, and pharmaceutical compositions comprising such triplex forming oligos (TFOs) are administered in circumstances in which excessive production, or production of aberrant protein, is a pathophysiologic basis of disease.

In another embodiment of the therapeutic methods of the present invention, a therapeutically effective amount of a pharmaceutical composition comprising an antibody (including fragment or derivative thereof) of the present invention is administered. As is well known, antibody compositions are administered, for example, to antagonize activity of MDZ3, MDZ4, MDZ7 or MDZ12, respectively, or to target therapeutic agents to sites of MDZ3, MDZ4, MDZ7 or MDZ12 presence and/or accumulation.

In another embodiment of the therapeutic methods of the present invention, a pharmaceutical composition comprising a non-antibody antagonist of MDZ3, MDZ4, MDZ7 or MDZ12 is administered. Antagonists of MDZ3, MDZ4, MDZ7 or MDZ12 can be produced using methods generally known in the art. In particular, purified MDZ3, MDZ4, MDZ7 or MDZ12 (MDZ12a, MDZ12bS, or MDZ12bL) can be used to screen libraries of pharmaceutical agents, often combinatorial libraries of small molecules, to identify those that specifically bind and antagonize at least one activity of MDZ3, MDZ4, MDZ7 or MDZ12, respectively.

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In other embodiments a pharmaceutical composition comprising an agonist of MDZ3, MDZ4, MDZ7 or MDZ12 is administered. Agonists can be identified using methods analogous to those used to identify antagonists.

5 In still other therapeutic methods of the present invention, pharmaceutical compositions comprising host cells that express MDZ3, MDZ4, MDZ7 or MDZ12, fusions, or fragments thereof can be administered. In such cases, the cells are typically autologous, so as to circumvent
10 xenogeneic or allotypic rejection, and are administered to complement defects in MDZ3, MDZ4, MDZ7 or MDZ12 production or activity.

In other embodiments, pharmaceutical compositions comprising the MDZ3, MDZ4, MDZ7 or MDZ12 proteins, nucleic
15 acids, antibodies, antagonists, and agonists of the present invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art according to
20 conventional pharmaceutical principles. The combination of therapeutic agents or approaches can act additively or synergistically to effect the treatment or prevention of the various disorders described above, providing greater therapeutic efficacy and/or permitting use of the
25 pharmaceutical compositions of the present invention using lower dosages, reducing the potential for adverse side effects.

TRANSGENIC ANIMALS AND CELLS

30

In another aspect, the invention provides transgenic cells and non-human organisms comprising MDZ3, MDZ4, MDZ7 or MDZ12 isoform nucleic acids, and transgenic

cells and non-human organisms with targeted disruption of the endogenous orthologue of the human MDZ3, MDZ4, MDZ7 or MDZ12 gene, respectively.

5 The cells can be embryonic stem cells or somatic cells. The transgenic non-human organisms can be chimeric, nonchimeric heterozygotes, and nonchimeric homozygotes.

DIAGNOSTIC METHODS

10 The nucleic acids of the present invention can be used as nucleic acid probes to assess the levels of MDZ3, MDZ4, MDZ7, MDZ12a or MDZ12b mRNA in disease tissues or cells, and antibodies of the present invention can be used to assess the expression levels of MDZ3, MDZ4, MDZ7, MDZ12a
15 or MDZ12b proteins in disease tissues or cells to diagnose a variety of diseases, including developmental disorders and cancer.

20 The following examples are offered for purpose of illustration, not limitation.

EXAMPLE 1

Identification and Characterization of cDNAs Encoding MDZ3 Proteins

25 Bioinformatic algorithms were applied to human genomic sequence data to identify putative exons. Using a graphical display particularly designed to facilitate computerized query of the resulting exon, eight exons were
30 identified as belonging to the same gene.

Marathon-Ready™ HeLa cell cDNA (Clontech Laboratories, Palo Alto, CA) was used as a substrate for standard RACE (rapid amplification of cDNA ends). Marathon-

ReadyTM cDNAs are adaptor-ligated double stranded cDNAs
suitable for 3' and 5' RACE. Chenchik et al., *BioTechniques*
21:526-532 (1996); Chenchik et al., *CLONTECHniques* X(1):5-8
(January 1995). RACE techniques are described, *inter alia*,
5 in the Marathon-ReadyTM cDNA User Manual (Clontech Labs.,
Palo Alto, CA, USA, March 30, 2000, Part No. PT1156-1
(PR03517)), Ausubel et al. (eds.), Short Protocols in
Molecular Biology : A Compendium of Methods from Current
Protocols in Molecular Biology, 4th edition (April 1999),
10 John Wiley & Sons (ISBN: 047132938X) and Sambrook et al.
(eds.), Molecular Cloning: A Laboratory Manual (3rd ed.),
Cold Spring Harbor Laboratory Press (2000) (ISBN:
0879695773), the disclosures of which are incorporated
herein by reference in their entireties.

15 Two overlapping RACE products were cloned that
together span 2.0 kilobases and that together appear to
contain the entire coding region of the gene to which the
exons contribute; for reasons described below, we termed
this cDNA MDZ3.

20 The MDZ3 cDNA was sequenced on both strands using
a MegaBaceTM sequencer (Molecular Dynamics, Inc., Sunnyvale,
CA, USA). Sequencing both strands provided us with the
exact chemical structure of the cDNA, which is shown in FIG.
3 and further presented in the SEQUENCE LISTING as SEQ ID
25 NO: 1, and placed us in actual physical possession of the
entire set of single-base incremented fragments of the
sequenced clone, starting at the 5' and 3' termini.

MDZ3 cDNA was sent to the American Type Culture
Collection (ATCC) for deposit on August 1, 2001, received by
30 ATCC on August 2, 2001, and accorded accession number
_____.

As shown in FIG. 3, the MDZ3 cDNA spans 1981 nucleotides and contains an open reading frame from nucleotide 311 through and including nt 1945 (inclusive of termination codon), predicting a protein of 544 amino acids with a (posttranslationally unmodified) molecular weight of 61.4 kD. The open reading frame appears full length with in-frame 5' stop codons, a methionine start codon and a stop codon.

BLAST query of genomic sequence identified one BAC, spanning 14 kb, that constitute the minimum set of clones encompassing the cDNA sequence. Based upon the known origin of the BAC (GenBank accession number AC005020.5), the MDZ3 gene can be mapped to human chromosome 7q22.1.

Comparison of the cDNA and genomic sequences identified 8 exons. Exon organization is listed in Table 1.

Table 1 MDZ3 Exon Structure			
Exon no.	cDNA range	genomic range	BAC accession
1	1-52	78844-78793	AC005020.5
2	53-179	77284-77158	
3	180-264	76761-76677	
4	265-697	76248-75816	
5	698-899	74436-74235	
6	900-991	73260-73169	
7	992-1115	71752-71629	
8	1116-1981	66618-65753	

FIG. 2 schematizes the exon organization of the MDZ3 clone.

At the top is shown the bacterial artificial chromosome (BAC), with GenBank accession number, that spans the MDZ3 locus.

As shown in FIG. 2, MDZ3, encoding a protein of 544 amino acids, is comprised of exons 1 - 8. Predicted molecular weight of the protein, prior to any post-translational modification, is 61.4 kD.

As further discussed in the examples herein, expression of MDZ3 was assessed using RT-PCR. RT-PCR product for MDZ3 was clearly produced from brain, testis, heart and bone marrow, but not from lung, liver, or skeletal muscle.

The sequence of the MDZ3 cDNA was used as a BLAST query into the GenBank nr and dbEst databases. The nr database includes all non-redundant GenBank coding sequence translations, sequences derived from the 3-dimensional structures in the Brookhaven Protein Data Bank (PDB), sequences from SwissProt, sequences from the protein information resource (PIR), and sequences from protein research foundation (PRF). The dbEst (database of expressed sequence tags) includes ESTs, short, single pass read cDNA (mRNA) sequences, and cDNA sequences from differential display experiments and RACE experiments. BLAST search identified two human ESTs (BE540085, AW892105), multiple mouse and rat ESTs, one EST from bovine (BE750886) and one from pig (BF079982) as having sequence closely related to MDZ3.

Globally, human MZD3 resembles a 604 amino acid residue hypothetical zinc finger protein, KIAA0426, at 36 % amino acid identity and 47 % amino acid similarity over 560 amino acids.

Motif searches using Pfam (<http://pfam.wustl.edu>), SMART (<http://smart.embl-heidelberg.de>), and PROSITE pattern and profile databases (<http://www>).

expasy.ch/prosite), identified several known domains shared with SCAN box containing Kruppel family zinc-finger proteins.

FIG. 1 shows the domain structure of MDZ3 and the alignment of SCAN box and KRAB domain in MDZ3 with similar motifs.

As schematized in FIG. 1, the newly isolated gene product shares certain protein domains and an overall structural organization with SCAN box- containing Kruppel family zinc-finger proteins. The shared structural features strongly imply that MDZ3 plays a role similar to that of other SCAN box- containing Kruppel family zinc-finger proteins as regulators of gene expression, and participates in protein-protein interactions with other transcription modulators. Thus MDZ3 is a clinically useful diagnostic marker and potential therapeutic agent for a ranges of diseases, including developmental disorders and cancer.

Possession of the genomic sequence permitted search for promoter and other control sequences for the MDZ3 gene.

A putative transcriptional control region, inclusive of promoter and downstream elements, was defined as 1 kb around the transcription start site, itself defined as the first nucleotide of the MDZ3 cDNA clone. The region, drawn from sequence of BAC AC005020.5, has the sequence given in SEQ ID NO: 24, which lists 1000 nucleotides before the transcription start site.

Transcription factor binding sites were identified using a web based program (<http://motif.genome.ad.jp/>), including two binding site for sex-determining region Y gene product (SRY, 474-480 bp and 482-488), for myoblast determining factor (961-970 bp), and for homeo domain factor

Nkx-2.5/Csx, tinman homolog (589-597 bp, with numbering according to SEQ ID NO: 24), amongst others.

We have thus identified a newly described human gene, MDZ3, which shares certain protein domains and an overall structural organization with SCAN box containing Kruppel family zinc-finger proteins. The shared structural features strongly imply that the MDZ3 protein plays a role similar to the other SCAN box containing Kruppel family zinc-finger proteins. It likely functions as a regulator of gene expression and participates in protein-protein interactions with other transcription modulators; thus, the MDZ3 proteins and nucleic acids are clinically useful diagnostic markers and potential therapeutic agents for a range of diseases, including developmental disorders and cancer.

EXAMPLE 2

Identification and Characterization of cDNAs Encoding MDZ4 Proteins

Predicating our gene discovery efforts on use of genome-derived single exon probes and hybridization to genome-derived single exon microarrays – an approach that we have previously demonstrated will readily identify novel genes that have proven refractory to mRNA-based identification efforts – we identified an exon in raw human genomic sequence that is particularly expressed in human bone marrow, brain, heart, HeLa, adult liver, fetal liver, lung, placenta and prostate.

Briefly, bioinformatic algorithms were applied to human genomic sequence data to identify putative exons. Each of the predicted exons was amplified from genomic DNA, typically centering the putative coding sequence within a

larger amplicon that included flanking noncoding sequence. These genome-derived single exon probes were arrayed on a support and expression of the bioinformatically predicted exons assessed through a series of simultaneous two-color hybridizations to the genome-derived single exon microarrays.

The approach and procedures are further described in detail in Penn et al., "Mining the Human Genome using Microarrays of Open Reading Frames," *Nature Genetics* 26:315-318 (2000); commonly owned and copending U.S. patent application nos. 09/864,761, filed May 23, 2001, 09/774,203, filed January 29, 2001, and 09/632,366, filed August 3, 2000, the disclosures of which are incorporated herein by reference in their entireties.

Using a graphical display particularly designed to facilitate computerized query of the resulting exon-specific expression data, as further described in commonly owned and copending U.S. patent application commonly owned and copending U.S. patent application nos. 09/864,761, filed May 23, 2001, and 09/774,203, filed January 29, 2001, the disclosures of which are incorporated herein by reference in their entireties, two exons were identified that are expressed in all the human tissues tested; subsequent analysis revealed that the two exons belong to the same gene.

Table 2 summarizes the microarray expression data obtained using genome-derived single exon probes corresponding to exons 2 and 3. Each probe was completely sequenced on both strands prior to its use on a genome-derived single exon microarray; sequencing confirmed the exact chemical structure of each probe. An added benefit of sequencing is that it placed us in possession of a set of single base-incremented fragments of the sequenced nucleic

acid, starting from the sequencing primer's 3' OH. (Since the single exon probes were first obtained by PCR amplification from genomic DNA, we were of course additionally in possession of an even larger set of single base incremented fragments of each of the single exon probes, each fragment corresponding to an extension product from one of the two amplification primers.)

Signals and expression ratios are normalized values measured and calculated as further described in commonly owned and copending U.S. patent application nos. 09/864,761, filed May 23, 2001, 09/774,203, filed January 29, 2001, and 09/632,366, filed August 3, 2000, the disclosures of which are incorporated herein by reference in their entireties.

Table 2 Expression Analysis Genome-Derived Single Exon Microarray				
	Ampl_31808 (exon_2)		Ampl_9581 (exon_3)	
	Signal	Expression ratio	Signal	Expression ratio
ADULT LIVER	n/d	n/d	1.4	-1.04
BONE MARROW	1.32	-1.69	n/d	n/d
BRAIN	1.43	-1.63	1.75	n/d
FETAL LIVER	2.17	-1.11	1.07	-1.16
HEART	1.78	-1.22	1.22	-1.07
HELA	2.04	-1.12	1.75	1.01
LUNG	0.78	n/d	n/d	n/d
PLACENTA	1.23	-2.54	1.61	1
PROSTATE	1.55	n/d	n/d	n/d

As shown in Table 2, significant expression of

exons 2 and 3 was seen only in human bone marrow, brain, heart, hela, adult liver, fetal liver, lung, placenta and prostate.

Marathon-Ready™ placenta cDNA (Clontech Laboratories, Palo Alto, CA, USA) was used as a substrate for standard RACE (rapid amplification of cDNA ends) to obtain a cDNA clone that spans 1.3 kilobases and appears to contain the entire coding region of the gene to which the exon contributes; for reasons described below, we termed this cDNA MDZ4. Marathon-Ready™ cDNAs are adaptor-ligated double stranded cDNAs suitable for 3' and 5' RACE. Chenchik et al., *BioTechniques* 21:526-532 (1996); Chenchik et al., *CLONTECHniques* X(1):5-8 (January 1995). RACE techniques are described, *inter alia*, in the Marathon-Ready™ cDNA User Manual (Clontech Labs., Palo Alto, CA, USA, March 30, 2000, Part No. PT1156-1 (PR03517)), Ausubel et al. (eds.), Short Protocols in Molecular Biology : A Compendium of Methods from Current Protocols in Molecular Biology, 4th edition (April 1999), John Wiley & Sons (ISBN: 047132938X) and Sambrook et al. (eds.), Molecular Cloning: A Laboratory Manual (3rd ed.), Cold Spring Harbor Laboratory Press (2000) (ISBN: 0879695773), the disclosures of which are incorporated herein by reference in their entireties.

The MDZ4 cDNA was sequenced on both strands using a MegaBace™ sequencer (Molecular Dynamics, Inc., Sunnyvale, CA, USA). Sequencing both strands provided us with the exact chemical structure of the cDNA, which is shown in FIG. 6 and further presented in the SEQUENCE LISTING as SEQ ID NO: 3027, and placed us in actual physical possession of the entire set of single-base incremented fragments of the sequenced clone, starting at the 5' and 3' termini.

MDZ4 cDNA was sent for deposit to the American Type Culture Collection on August 1, 2001, received at ATCC

on August 2, 2001, and accorded accession number

As shown in FIG. 6, the MDZ4 cDNA spans 1329 nucleotides and contains an open reading frame from nucleotide 142 through and including nt 1311 (inclusive of termination codon), predicting a protein of 389 amino acids with a (posttranslationally unmodified) molecular weight of 44.9 kD. The clone appears full length, with the reading frame opening with a methionine and terminating with a stop codon.

BLAST query of genomic sequence identified one PAC, spanning 128 kb, that constitutes the minimum set of clones encompassing the cDNA sequence. Based upon the known origin of the PAC (GenBank accession numbers Z98745.1), the MDZ4 gene can be mapped to human chromosome 6p21.3-22.2.

Comparison of the cDNA and genomic sequences identified 4 exons. Exon organization is listed in Table 3.

Table 3 MDZ4 Exon Structure			
Exon no.	cDNA range	genomic range	PAC accession
1	1-64	62122-62059	Z98745.1
2	65-549	55003-54519	
3	550-697	54267-54120	
4	698-1328	53738-53107	

FIG. 5 schematizes the exon organization of the MDZ4 clone.

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40 % amino acid identity and 55 % amino acid similarity over
447 a.a.), ZNF193 protein (GenBank Accession number: O15535;
54 % amino acid identity and 68 % amino acid similarity over
387 a.a.) and ZNF232 protein (GenBank Accession number:
5 Q9UNY5; 47 % amino acid identity and 59 % amino acid
similarity over 387 a.a.).

Motif searches using Pfam (<http://pfam.wustl.edu>),
SMART (<http://smart.embl-heidelberg.de>), and PROSITE pattern
and profile databases (<http://www.expasy.ch/prosite>),
10 identified several known domains shared with SCAN box
containing Kruppel family zinc-finger proteins.

FIG. 4 shows the domain structure of MDZ4,
including the overall structure of MDZ4 and the alignment of
the SCAN box in MDZ4 with similar motifs.

15 As schematized in FIG. 4, the newly isolated gene
product shares certain protein domains and an overall
structural organization with SCAN box containing Kruppel
family zinc-finger proteins. The shared structural features
strongly imply that MDZ4 plays a role similar to that of
20 SCAN box containing Kruppel family zinc-finger proteins as a
potential transcription regulator, and is likely to
participate in protein-protein interactions with other
transcription modulators. Thus, MDZ4 is a clinically useful
diagnostic markers and potential therapeutic agents for a
25 variety of diseases, including developmental disorders and
cancer.

Possession of the genomic sequence permitted
search for promoter and other control sequences for the MDZ4
gene.

30 A putative transcriptional control region,
inclusive of promoter and downstream elements, was defined
as 1 kb around the transcription start site, itself defined
as the first nucleotide of the MDZ4 cDNA clone. The region,

drawn from sequence of PAC Z98745.1, has the sequence given in SEQ ID NO: 3046, which lists 1000 nucleotides before the transcription start site.

Transcription factor binding sites were identified using a web based program (<http://motif.genome.ad.jp/>), including a binding site for CdxA (771-777 bp) and for cap signal for transcription initiation (984-991 bp, with numbering according to SEQ ID NO: 3046), amongst others.

We have thus identified a newly described human gene, MDZ4, which shares certain protein domains and an overall structural organization with SCAN box containing Kruppel family zinc-finger proteins. The shared structural features strongly imply that the MDZ4 protein plays a role similar to SCAN box containing Kruppel family zinc-finger proteins, as a potential transcription regulator, and is likely to participate in protein-protein interactions with other transcription modulators. Thus MDZ4 nucleic acids and proteins are clinically useful diagnostic markers and potential therapeutic agents for a variety of diseases, including developmental disorders and cancer.

EXAMPLE 3

Identification and Characterization of cDNAs Encoding MDZ7 Proteins

Bioinformatic algorithms were applied to human genomic sequence data to identify putative exons. Using a graphical display particularly designed to facilitate computerized query of the resulting exon, four exons were identified as belonging to the same gene.

Marathon-Ready™ placenta cDNA (Clontech Laboratories, Palo Alto, CA, USA) was used as a substrate for standard RACE (rapid amplification of cDNA ends).

Marathon-Ready™ cDNAs are adaptor-ligated double stranded
cDNAs suitable for 3' and 5' RACE. Chenchik et al.,
BioTechniques 21:526-532 (1996); Chenchik et al.,
CLONTECHniques X(1):5-8 (January 1995). RACE techniques are
5 described, *inter alia*, in the Marathon-Ready™ cDNA User
Manual (Clontech Labs., Palo Alto, CA, USA, March 30, 2000,
Part No. PT1156-1 (PR03517)), Ausubel et al. (eds.), Short
Protocols in Molecular Biology : A Compendium of Methods
from Current Protocols in Molecular Biology, 4th edition
10 (April 1999), John Wiley & Sons (ISBN: 047132938X) and
Sambrook et al. (eds.), Molecular Cloning: A Laboratory
Manual (3rd ed.), Cold Spring Harbor Laboratory Press (2000)
(ISBN: 0879695773), the disclosures of which are
incorporated herein by reference in their entireties.

15 Three overlapping RACE products were cloned that
together contain the complete sequence of MDZ7, a cDNA clone
that collectively spans 2.2 kilobases and appears to contain
the entire coding region of the gene to which the exon
contributes; for reasons described below, we termed this
20 cDNA MDZ7.

The MDZ7 cDNA was sequenced on both strands using
a MegaBace™ sequencer (Molecular Dynamics, Inc., Sunnyvale,
CA, USA). Sequencing both strands provided us with the
exact chemical structure of the cDNA, which is shown in FIG.
25 9 and further presented in the SEQUENCE LISTING as SEQ ID
NO: 4407, and placed us in actual physical possession of the
entire set of single-base incremented fragments of the
sequenced clone, starting at the 5' and 3' termini.

In order to assess expression in a variety of
30 tissues, we generated a pair of PCR primers to analyze the
expression pattern of the human MDZ7 gene in standard RT-PCR
experiments (Sambrook et al., *Molecular cloning: 3rd*
edition, 2001). RT-PCR product for MDZ7 was produced from

testis. These experiments placed us in possession of a near complete set of fragments of the template.

MDZ7 cDNA was sent for deposit to the American Type Culture Collection on August 1, 2001, received at ATCC
5 August 2, 2001, and accorded accession number _____.

As shown in FIG. 9, the MDZ7 cDNA spans 2198 nucleotides and contains an open reading frame from nucleotide 663 through and including nt 1409 (inclusive of termination codon), predicting a protein of 248 amino acids
10 with a (posttranslationally unmodified) molecular weight of 28.8 kD. The open reading frame appears full length with an in-frame 5' stop codon, a methionine start codon and a stop codon before a 3' poly-A tail.

BLAST query of genomic sequence identified one
15 BAC, spanning 121 kb, that constitute the minimum set of clones encompassing the cDNA sequence. Based upon the known origin of the BAC (GenBank accession numbers AC002310.1), the MDZ7 gene can be mapped to human chromosome 16p11.2.

Comparison of the cDNA and genomic sequences
20 identified 4 exons. Exon organization is listed in Table 4.

Table 4 MDZ7 Exon Structure			
Eexon no.	cDNA range	genomic range	PAC accession
1	1-396	1531-1926	AC002310.1
2	397-525	2497-2625	
3	526-1458	4304-5236	
4	1459-2198	6181-6920	

FIG. 8 schematizes the exon organization of the MDZ7 clone.

At the top is shown the bacterial artificial
5 chromosome (BAC), with GenBank accession numbers, that spans
the MDZ7 locus. As shown in FIG. 8, MDZ7 is comprised of
four exons and encodes a protein of 248 amino acids.
Predicted molecular weight of the MDZ7 protein, prior to any
post-translational modification, is 28.8 kD.

10 As further discussed in the examples herein,
expression of MDZ7 was assessed using RT-PCR. RT-PCR
analysis of showed MDZ7 expression only in testes, but not
in brain, lung, liver, kidney, skeletal muscle, heart, whole
fetus, or Hela cells.

15 The sequence of the MDZ7 cDNA was used as a BLAST
query into the GenBank nr and dbEst databases. The nr
database includes all non-redundant GenBank coding sequence
translations, sequences derived from the 3-dimensional
structures in the Brookhaven Protein Data Bank (PDB),
20 sequences from SwissProt, sequences from the protein
information resource (PIR), and sequences from protein
research foundation (PRF). The dbEst (database of expressed
sequence tags) includes ESTs, short, single pass read cDNA
(mRNA) sequences, and cDNA sequences from differential
25 display experiments and RACE experiments. BLAST search
identified multiple human ESTs as having sequence closely
related to MDZ7.

Motif searches using Pfam (<http://pfam.wustl.edu>),
SMART (<http://smart.embl-heidelberg.de>), and PROSITE pattern
30 and profile databases (<http://www.expasy.ch/prosite>),
identified several Kruppel family zinc-finger motifs.

FIG. 7 shows the domain structure of MDZ7.

As schematized in FIG. 7, the newly isolated MDZ7

is mainly composed of seven tandemly arrayed Kruppel-type (C2H2) zinc finger repeats. Such a structure implies that MDZ7 is likely to function in sequence-specific DNA binding and impart a regulatory effect on specific gene expression.

5 Thus MDZ7 nucleic acids and proteins are clinically useful diagnostic markers and potential therapeutic agents for a variety of diseases, including developmental disorders and cancer.

Possession of the genomic sequence permitted
10 search for promoter and other control sequences for the MDZ7 gene.

A putative transcriptional control region, inclusive of promoter and downstream elements, was defined as 1 kb around the transcription start site, itself defined
15 as the first nucleotide of the MDZ7 cDNA clone. The region, drawn from sequence of BAC AC002310.1, has the sequence given in SEQ ID NO: 4420, which lists 1000 nucleotides before the transcription start site.

Transcription factor binding sites were identified
20 using a web based program (<http://motif.genome.ad.jp/>), including a binding site for cap signal for transcription initiation (846-853 bp, with numbering according to SEQ ID NO: 4420), amongst others.

We have thus identified a newly described human
25 gene, MDZ7, which contains seven Kruppel family zinc-finger motifs. The structural features strongly imply that the MDZ7 protein plays a role similar to other Kruppel family zinc-finger proteins, as a potential transcription regulator. Thus, MDZ7 nucleic acids and proteins are clinically useful
30 diagnostic markers and potential therapeutic agents for a variety of diseases, including developmental disorders and cancer.

were used to PCR out the 5' end of the gene,
oligonucleotides OL311 (5'-GGTCACCTGTACGCTCCTCTCCATGTCTCTTC-
3') [SEQ ID NO:7040] and OL312 (5'-
CTGTTTGGCTTCCGACCTGCTCCTCACC-3') [SEQ ID NO:7041] were used
5 to PCR out the 3' end of the gene. The PCR fragments are
sequenced using a MegaBACE™ sequencer. The final contig of
the sequences revealed a 1.78 kb novel gene with a 1.45 kb
ORF.

To subclone MDZ12a into a cloning vector, the RACE
10 product generated with oligonucleotides OL612 and OL638 (5'-
CCACCATGTGGCTGGGGACTTCAGGGAAGAGTGGGTAC-3') [SEQ ID NO:7042]
against human heart marathon-ready cDNA (Clontech) was
ligated and T/A cloned into pGem-Teasy vector (Promega
Corp.). Individual clones were picked and inserts
15 sequenced. A second MDZ12 transcript, with the insertion of
an extra exon with 66 nucleotides between exons 2 and 3 of
MDZ12a, was identified. This transcript is named MDZ12b. The
insertion of the extra exon introduces an early stop codon,
reducing the initial ORF to 44 amino acids. However, the
20 MDZ12b transcript does still contain another ORF downstream
with 332 amino acids.

The MDZ12a cDNA was sequenced on both strands
using a MegaBace™ sequencer (Molecular Dynamics, Inc.,
Sunnyvale, CA, USA). Sequencing both strands provided us
25 with the exact chemical structure of the cDNA, which is
shown in FIG. 12 and further presented in the SEQUENCE
LISTING as SEQ ID NO: 5770, and placed us in actual physical
possession of the entire set of single-base incremented
fragments of the sequenced clone, starting at the 5' and 3'
30 termini.

MDZ12a and MDZ12b cDNA were deposited together in
a single tube at the American Type Culture Collection; the
deposit was sent to ATCC for deposit on August 1, 2001,

received at ATCC August 2, 2001, and accorded accession number _____.

As shown in FIG. 12, the MDZ12a cDNA spans 1780 nucleotides and contains an open reading frame from
5 nucleotide 127 through and including nt 1578 (inclusive of termination codon), predicting a protein of 483 amino acids with a (posttranslationally unmodified) molecular weight of 55.1 kD. The open reading frame appears full length with in-frame 5' stop codons, a methionine start codon and a stop
10 codon.

As shown in FIG. 13, the MDZ12b cDNA spans at least 1518 nucleotides and contains two open reading frames. The shorter ORF, MDZ12bS, is from nucleotide 1 through and including nt 135 (inclusive of termination codon),
15 predicting a polypeptide of 44 amino acids. While the longer ORF, MDZ12bL, is from nucleotide 520 through and including nt 1518 (inclusive of termination codon), predicting a polypeptide of 332 amino acids with a (posttranslationally unmodified) molecular weight of 38.2 kD.

20 BLAST query of genomic sequence identified one BAC, that constitute the minimum set of clone encompassing the cDNA sequence. Based upon the known origin of the BAC (GenBank accession numbers AC018946.5), the MDZ12 gene can be mapped to human chromosome 15q26.1.

25 Comparison of the cDNA and genomic sequences identified 4 exons for MDZ12a. Exon organization is listed in Table 5. The additional exon found in MDZ12b is numbered as 2'.

Table 5
Exon Structure of the MDZ12 gene

used as BLAST queries into the GenBank nr and dbEst
databases. The nr database includes all non-redundant
GenBank coding sequence translations, sequences derived from
the 3-dimensional structures in the Brookhaven Protein Data
Bank (PDB), sequences from SwissProt, sequences from the
protein information resource (PIR), and sequences from
protein research foundation (PRF). The dbEst (database of
expressed sequence tags) includes ESTs, short, single pass
read cDNA (mRNA) sequences, and cDNA sequences from
differential display experiments and RACE experiments. BLAST
search identified multiple human ESTs as having sequence
closely related to MDZ12.

Motif searches using Pfam (<http://pfam.wustl.edu>),
SMART (<http://smart.embl-heidelberg.de>), and PROSITE
pattern and profile databases
(<http://www.expasy.ch/prosite>), identified several known
domains shared with KRAB domain containing Kruppel family
zinc-finger proteins for MDZ12a and Kruppel family zinc-
fingers for MDZ12bL.

FIG. 10A shows the domain structure of MDZ12,
including the overall structure of MDZ12a and MDZ12bL, with
FIG. 10B showing the alignment of KRAB domain in MDZ12a with
similar motifs.

As schematized in FIGS. 10A and 10B, the newly
isolated MDZ12a contains a partial KRAB motif as well as
twelve copies of C2H2 zinc fingers. The MDZ12bL contains
twelve copies of C2H2 zinc fingers. Such features strongly
imply that MDZ12 plays a role as a potential transcription
regulator, and is likely to participate in protein-protein
interactions with other transcription modulators. Thus MDZ12
nucleic acids and proteins are clinically useful diagnostic
markers and potential therapeutic agents for a variety of
diseases, including developmental disorders and cancer.

Possession of the genomic sequence permitted search for promoter and other control sequences for the MDZ12 gene.

5 A putative transcriptional control region, inclusive of promoter and downstream elements, was defined as 1 kb around the transcription start site, itself defined as the first nucleotide of the MDZ12 cDNA clone. The region, drawn from sequence of BAC AC018946.5, has the sequence given in SEQ ID NO: 5783, which lists 1000
10 nucleotides before the transcription start site.

Transcription factor binding sites were identified using a web based program (<http://motif.genome.ad.jp/>), including a binding site for signal transducers and activators of transcription (STATx, 837-845 bp) and for
15 GATA-binding factor 1 (GATA-1, 926-935 bp), with numbering according to SEQ ID NO: 5783), amongst others.

We have thus identified a newly described human gene, MDZ12, which shares certain protein domains and an overall structural organization with KRAB box containing
20 Kruppel family zinc-finger proteins. The shared structural features strongly imply that the MDZ12a and MDZ12bL proteins play a role similar to KRAB box containing Kruppel family zinc-finger proteins, as a potential transcription regulator, and is likely to participate in protein-protein
25 interactions with other transcription modulators. Thus MDZ12 is a clinically useful diagnostic marker and potential therapeutic agent for a variety of diseases, including developmental disorders and cancer.

30

EXAMPLE 5

RT-PCR analysis of expression of human MDZ3

RT-PCR analysis was used to determine the

expression pattern of the human MDZ3 gene. A forward primer (5'-GATGGCGGAAGCTCCTCAGC) [SEQ ID NO:7043] and a reverse primer (5'-AGTCCTGCGGTTCCACATAC) [SEQ ID NO:7044] derived from the middle of the MDZ3 gene were used in standard RT-PCR (Sambrook et al., Molecular Cloning (3rd ed.), 2001).
5 Templates for the PCRs were obtained from brain, liver, lung, bone marrow, heart, skeletal muscle, and testis and reactions were carried out according to the following schedule: 94°C, 20 seconds; 68°C 20 seconds; 72°C, 60
10 seconds, for 35 cycles). PCR products were separated on an agarose gel and visualized with a Typhoon™ fluorimager and ImageQuant™ software (Molecular Dynamics, Sunnyvale, CA). RT-PCR product for MDZ3 was produced from brain, testis, heart and bone marrow, but not from lung, liver, or skeletal
15 muscle (FIG. 14).

EXAMPLE 6

RT-PCR analysis of expression of human MDZ7

20 RT-PCR analysis was used to determine the expression pattern of the human MDZ7 gene. A forward primer (5'-TCAGATCTGTCGCTCCTTCA) [SEQ ID NO:7045] and a reverse primer (5'-GCAGTCTGAGCACGCGTAAG) [SEQ ID NO:7046] derived from the open reading frame of MDZ7 were used in standard
25 RT-PCRs (Sambrook et al., Molecular cloning: 3rd edition, 2001). Templates for the PCRs were obtained from whole fetus, liver, lung, kidney, heart, and testis and reactions were carried out according to the following schedule: 94°C, 20 seconds; 68°C 20 seconds; 72°C, 60 seconds, for 35
30 cycles). PCR products were separated on an agarose gel and visualized with a Typhoon™ fluorimager and ImageQuant™ software (Molecular Dynamics, Sunnyvale, CA). RT-PCR product for MDZ7 was only produced from testis (FIG. 15).

EXAMPLE 7

RT-PCR analysis of MDZ12 expression

5 To explore the potential function of the MDZ12
gene, the expression of MDZ12 gene in human tissues was
examined by PCR using marathon-ready cDNAs. Oligonucleotides
OL612 and OL638 were used to amplify both MDZ12a and MDZ12b
from human cDNAs of bone marrow, brain, heart, kidney,
10 liver, placenta, skeletal muscle, testis and Hela cells. The
PCR conditions were according to a touchdown PCR procedure.
The tubes containing the oligonucleotides, cDNA and Taq
polymerase were first incubated at 94°C for 15 seconds
followed by 72°C for 2 minutes, cycle 5 times. The tubes
15 were then incubated at 94°C for 15 seconds followed by 70°C
for 2 minutes, cycle 5 times. Finally the tubes were
incubated at 94°C for 15 seconds followed by 68°C for 2
minutes, cycle 25 times. The result of the expression
profile is shown in figure 16. The abundance of PCR product
20 indicates that MDZ12a is expressed in all tissue examined
with highest expression in brain, heart, skeletal muscle,
testis and Hela cells (Figure 16). MDZ12b, however, is
expressed with lower to much lower abundance compared with
MDZ12a in bone marrow, brain, heart, kidney, placenta,
25 skeletal muscle, testis and Hela cells with almost no
expression in liver.

EXAMPLE 8

30 Preparation and Labeling of Useful
Fragments of MDZ3, MDZ4, MDZ7 and MDZ12

Useful fragments of MDZ3, MDZ4, MDZ7 and MDZ12 are produced by PCR, using standard techniques, or solid phase chemical synthesis using an automated nucleic acid synthesizer. Each fragment is sequenced, confirming the exact chemical structure thereof.

The exact chemical structure of preferred fragments is provided in the attached SEQUENCE LISTING, the disclosure of which is incorporated herein by reference in its entirety. The following summary identifies the fragments whose structures are more fully described in the SEQUENCE LISTING:

	SEQ ID NO: 1	(nt, full length MDZ3 cDNA)
	SEQ ID NO: 2	(nt, cDNA ORF of MDZ3)
15	SEQ ID NO: 3	(aa, full length protein of MDZ3)
	SEQ ID NO: 4	(nt, nt 201 - 1721 of MDZ3)
	SEQ ID NO: 5	(nt, 5' UT portion of SEQ ID NO: 4)
	SEQ ID NO: 6	(nt, coding region of SEQ ID NO: 4)
20	SEQ ID NO: 7	(aa, aa 1 - 470, CDS entirely within SEQ ID NO: 6)
	SEQ ID NOs: 8 - 15	(nt, exon 1 - 8 from genomic sequence of MDZ3)
	SEQ ID NOs: 16 - 23	(nt, 500 bp genomic amplicon centered about exons 1 - 8 of MDZ3)
25	SEQ ID NO: 24	(nt, 1000 bp putative promoter of MDZ3)
	SEQ ID NOs: 25-1529	(nt, 17-mers scanning nt 201 - 1721 of human MDZ3)
	SEQ ID NOs: 1530 - 3026	(nt, 25-mers scanning nt 201 - 1721 of human MDZ3)
30	SEQ ID NO: 3027	(nt, full length MDZ4 cDNA)
	SEQ ID NO: 3028	(nt, cDNA ORF of MDZ4)
	SEQ ID NO: 3029	(aa, full length protein of MDZ4)

SEQ ID NOS: 5784 - 6364

(nt, 17-mers scanning nt 352 - 948 of human MDZ12)

SEQ ID NOs: 6365 - 6937

5 (nt, 25-mers scanning nt 352 - 948 of
human MDZ12)

SEQ ID NO: 6938 (nt, cDNA of MDZ12b)

SEQ ID NO: 6939 (aa, full length protein of MDZ12bS)

SEQ ID NO: 6940 (aa, full length protein of MDZ12bL)

10 SEQ ID NO: 6941 (nt, novel exon (nt 105 - 170 portion)
of MDZ12b)

SEQ ID NO: 6942 (aa, novel exon (before stop codon) of MDZ12b)

15 SEQ ID NO: 6943 (nt, 500 bp genomic amplicon centered about novel exon of MDZ12b)

SEQ ID NOS: 6944 - 6993

(nt, 17-mers scanning novel exon of human MDZ12b)

SEQ ID NOS: 6994 - 7035

20 (nt, 25-mers scanning novel exon of
human MDZ12b)

SEQ ID NO: 7036 (nt, oligo OL612)

SEQ ID NO: 7037 (nt, oligo OL613)

SEQ ID NO: 7038 (nt, oligo OL614)

25 SEQ ID NO: 7039 (nt, oligo OL615)

SEQ ID NO: 7040 (nt, oligo OL311)

SEQ ID NO: 7041 (nt, oligo OL312)

SEQ ID NO: 7042 (nt, oligo OL638)

SEQ ID NO: 7043 (nt, forward primer for MDZ3 RT PCR)

30 SEQ ID NO: 7044 (nt, reverse primer for MDZ3 RT PCR)

SEQ ID NO: 7045 (nt, forward primer for MDZ7 RT PCR)

SEQ ID NO: 7046 (nt, reverse primer for MDZ7 RT PCR)

Upon confirmation of the exact structure, each of the above-described nucleic acids of confirmed structure is recognized to be immediately useful as a MDZ3, MDZ4, MDZ7 or MDZ12-specific probe.

5 For use as labeled nucleic acid probes, the above-described MDZ3, MDZ4, MDZ7 or MDZ12 nucleic acids are separately labeled by random priming. As is well known in the art of molecular biology, random priming places the investigator in possession of a near-complete set of labeled
10 fragments of the template of varying length and varying starting nucleotide.

The labeled probes are used to identify the MDZ3, MDZ4, MDZ7 or MDZ12 gene on a Southern blot, and are used to measure expression of MDZ3, MDZ4, MDZ7 or MDZ12 mRNA on a
15 northern blot and by RT-PCR, using standard techniques.

EXAMPLE 9

Production of MDZ3, MDZ4, MDZ7 or MDZ12 Protein

20 The full length MDZ3, MDZ4, MDZ7, MDZ12a or MDZ12b cDNA clone is cloned into the mammalian expression vector pCDNA3.1/HISA (Invitrogen, Carlsbad, CA, USA), transfected into COS7 cells, transfectants selected with G418, and protein expression in transfectants confirmed by detection
25 of the anti-Xpress[™] epitope according to manufacturer's instructions. Protein is purified using immobilized metal affinity chromatography and vector-encoded protein sequence is then removed with enterokinase, per manufacturer's instructions, followed by gel filtration and/or HPLC.

30 Following epitope tag removal, MDZ3, MDZ4, MDZ7, MDZ12a, MDZ12bS or MDZ12bL protein is present at a concentration of at least 70%, measured on a weight basis with respect to total protein (i.e., w/w), and is free of

acrylamide monomers, bis acrylamide monomers, polyacrylamide and ampholytes. Further HPLC purification provides MDZ3, MDZ4, MDZ7, MDZ12a, MDZ12bS or MDZ12bL protein at a concentration of at least 95%, measured on a weight basis with respect to total protein (i.e., w/w).

EXAMPLE 10

Production of Anti- MDZ3, MDZ4, MDZ7 or MDZ12 Antibody

Purified proteins prepared as in Example 6 are separately conjugated to carrier proteins and used to prepare murine monoclonal antibodies by standard techniques.

Initial screening with the unconjugated purified proteins, followed by competitive inhibition screening using peptide fragments of the MDZ3, MDZ4, MDZ7, MDZ12a, MDZ12bS or MDZ12bL, identifies monoclonal antibodies with specificity for MDZ3, MDZ4, MDZ7, MDZ12a, MDZ12bS or MDZ12bL.

Diseases that map to the MDZ3 chromosomal region are shown in Table 6. Mutation of the MDZ3 gene contributes to one or more of these conditions.

Table 6 Diseases mapped to human chromosome 7q22.1 (MDZ3 region)		
mim_num	disease	chromosomal location
209850	Autism, susceptibility to	7q
145290	Hyperreflexia	7q
603511	Limb-girdle muscular dystrophy-1D	7q

EXAMPLE 14

MDZ4 Disease Associations

Diseases that map to the MDZ4 chromosomal region are shown in Table 7. Mutation of the MDZ4 gene contributes to one or more of these conditions.

Table 7 Diseases mapped to human chromosome 6p21.3 (MDZ4 region)		
mim_num	disease	chromosomal location
106300	Ankylosing spondylitis	<u>6p21.3</u>
108800	Atrial septal defect, secundum type	<u>6p21.3</u>
146520	Hypotrichosis simplex of scalp	<u>6p21.3</u>
222100	Insulin-dependent diabetes mellitus-1	<u>6p21.3</u>
137100	Immunoglobulin A deficiency	<u>6p21.3</u>
146850	Immune suppression to streptococcal antigen	<u>6p21.3</u>
604809	Panbronchiolitis, diffuse	<u>6p21.3</u>

167250	Paget disease of bone	<u>6p21.3</u>
177900	Psoriasis susceptibility 1	<u>6p21.3</u>
179450	Ragweed sensitivity	<u>6p21.3</u>
150270	Laryngeal adductor paralysis	6p21.3 - p21.2

EXAMPLE 15

MDZ7 Disease Associations

5

Diseases that map to the MDZ7 chromosomal region are shown in Table 8. Mutation of the MDZ7 gene contributes to one or more of these conditions.

Table 8 Diseases mapped to chromosome 16p11.2 (MDZ7 region)		
mim_num	disease	chromosomal location
157700	Mitral valve prolapse, familial	16p12.1-p11.2
602066	Convulsions, infantile and paroxysmal choreoathetosis	16p12-q12
266600	Inflammatory bowel disease-1	16p12-q13
186580	Arthrocutaneouveal granulomatosis	16p12-q21
128200	Paroxysmal kinesigenic choreoathetosis	16p11.2-q12.1

10

EXAMPLE 16

MDZ12 Disease Associations

15

Diseases that map to the MDZ12 chromosomal region are shown in Table 9. Mutation of the MDZ12 gene contributes to one or more of these conditions.

Table 9:

Diseases mapped to chromosome 15q26.1 (MDZ12 region).		
mim_num	disease	chromosomal location
604329	Hypertension, essential, susceptibility to, 2	15q
214900	Cholestasis-lymphedema syndrome	15q
604416	Pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA syndrome)	15q24-q26.1
603813	Hypercholesterolemia, familial, autosomal recessive, 1	15q25-q26
600318	Insulin-dependent diabetes mellitus-3	15q26
166800	Otosclerosis 1	15q26.1-qter

All patents, patent publications, and other published references mentioned herein are hereby incorporated by reference in their entireties as if each had been individually and specifically incorporated by reference herein. While preferred illustrative embodiments of the present invention are described, one skilled in the art will appreciate that the present invention can be practiced by other than the described embodiments, which are presented for purposes of illustration only and not by way of limitation. The present invention is limited only by the claims that follow.